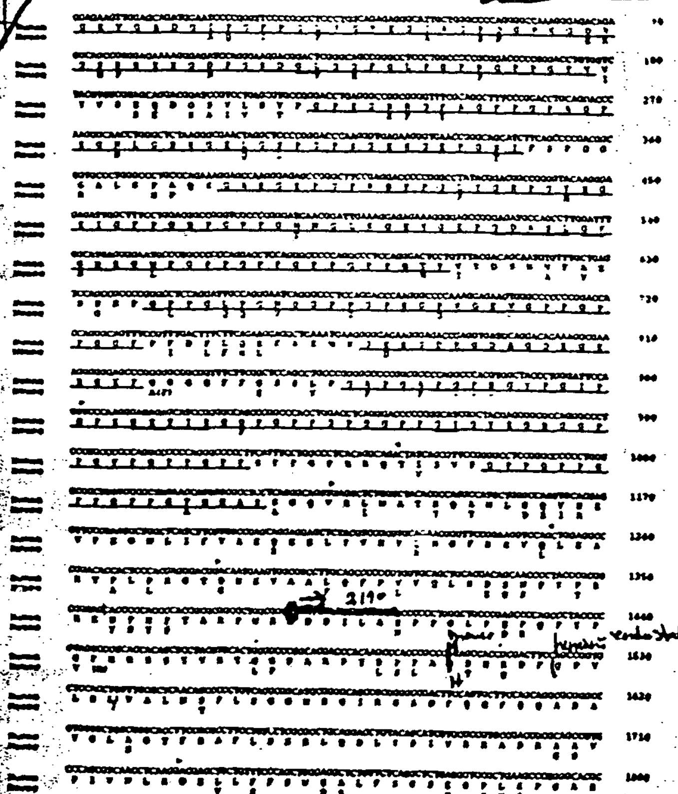
EXHIBIT C

Project No. 414
Book No. 003
TITLE Primers for Human Endostatin PCR

From Page No. [redacted]

Human α1 (XVIII) Collagen Gene Sequence Genbank Accession # L22548
The actual sequence alignment with mouse came from paper
Cloned cDNA & Genomic DNA Encoding Human Type XVIII Collagen
And Localization of the α1 (XVIII) Collagen Gene to Mouse Chromosome
10 and Human Chromosome 21 Suk R, DH, et al Genomics 19 pg 494-499 (1994)

Book1003 pg. 110 ... ET AL [redacted]



Primers made are highlighted in hot pink for endostatin

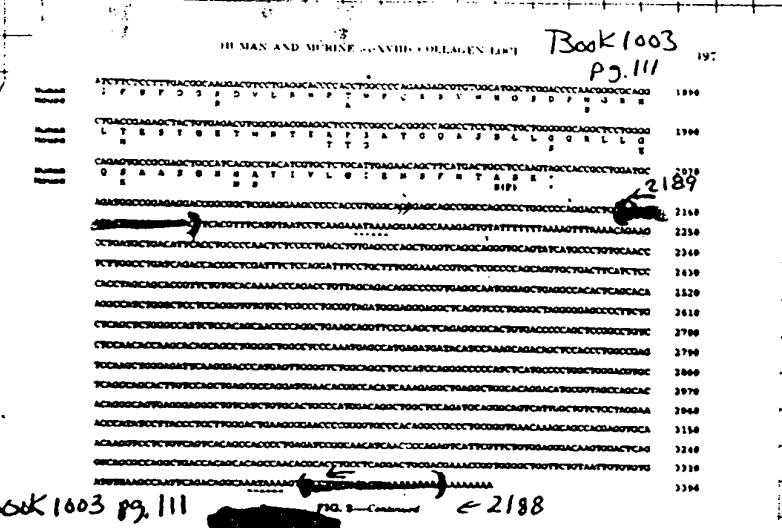
To Page No. 111

Witnessed & Understood by me, [signature] | Date [redacted] | Invented by | Recorded by [signature: Sandra Phipps] | Date [redacted]

TITLE Primers for Human Endostatin PCR

Project No. 414
Book No. 1003

From Page No. 110



Book 1003 pg. 111

Book 1003 pg. 111

FIG. 2—Continued

signals (AATAAA) about 1 kb apart, we tested whether the difference between the 5.0- and 6.0-kb transcripts was due to the utilization of alternative polyA) sites.

Oligo Request Form

Name: Sandrina Phipps | Project Code: 414 | Date: [redacted]

Length of Oligo: 22 Antisense

Purification: ☐ Unpurified ☐ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*

Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)

Sequence written 5' to 3' in triplets:

CTA TAC AGG AAA GTA TGG CAG C ___ ___

Total #G 5 #A 8 #T 4 #C 4

2189 0.7 mg/ml

Purpose: PCR GENE SYNTHESIS

SSP [redacted]

Book 1003 pg. 111

Oligo Request Form

Name: Sandrina Phipps | Project Code: 414 | Date: [redacted]

Length of Oligo: Antisense

Purification: ☐ Unpurified ☐ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*

Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)

Sequence written 5' to 3' in triplets:

TTT TTT TTT CAG TGT AAA AGG TC ___ ___

Total #G 4 #A 5 #T 12 #C 2 2188 (2.6 mg/ml)

Purpose: PCR GENE SYNTHESIS

SSP [redacted]

Book 1003 pg. 111

To Page No. 112

Witnessed & Understood by me, Lynn M. Clark

Date [redacted]

Invented by Sandrina Phipps

Recorded by

Date [redacted]

Project No. 414
Book No. 1003 TITLE Primers for Human Endostatin

From Page No. 111 [redacted]

Oligo Request Form

Name: Sandrina Phipps	Project Code: 414	Date: [redacted]

Length of Oligo: 19 sense

Purification: ☐ Unpurified ☐ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*

Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)

Sequence written 5' to 3' in triplets:

CAG ATG ACA TCC TGG CCA G

Total #G 5 #A 5 #T 3 #C 6 2190 2.3mg/ml

Purpose: PCR gene synthesis Book 1003 pg. 112 SSP [redacted]

Oligo Request Form

Name: Sandrina Phipps	Project Code: 414	Date: [redacted]

Length of Oligo: 17 A.S.

Purification: ☐ Unpurified ☐ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*

Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)

Sequence written 5' to 3' in triplets:

III III III III III II

Total #G ___ #A ___ #T 12 #C ___ 2187 2.4mg/ml

Purpose: PCR Book 1003 pg. 112 SSP [redacted]

[redacted]

Sandrina Phipps

[redacted]

TCID 50 worksheet for A inBgl X SSP1-98 from page 38. Calculated titer 5.18×10^8 PFU/ml. This is very low titer compared to particles of 1.5×10^{11} particles/ml. It also was identified by PCR that this virus does not appear to have the AS site primer does not amplify to product. I probably will not use this virus.

Dilutions	Number of Infected Wells	Number of Uninfected Wells	Total Number Infected	Total Numbe Uninfected	Percent Total Infect	Above 50%	Percent Above 50%	Percent Below 50%
1.00E-06	12	0	21	0	100.00%	TRUE	0.00%	0.00
1.00E-07	7	5	9	5	64.29%	TRUE	64.29%	0.00
1.00E-08	1	11	2	16	11.11%	FALSE	0.00%	11.11
1.00E-09	1	11	1	27	3.57%	FALSE	0.00%	0.00
1.00E-10	0	12	0	39	0.00%	FALSE	0.00%	0.00
1.00E-11	0	12	0	51	0.00%	FALSE	0.00%	0.00
1.00E-12	0	12	0	63	0.00%	FALSE	0.00%	0.00

Book 1003 page 112 SSP [redacted]

# of Wells	12						0.64285714	0.1111111
mls/well	0.02							
Prop. Dist.	0.268656716							
Log TCID50	-7.268656716							
TCID50	5.39E-08							
1/TCID50	1.86E+07			PFU/Ml	AVG PFU/M	Titer PFU/ML		
			#2	1.94E+08	3.44E+08			
TCID50/ml	9.28E+08			4.93E+08		5.18E+08		
			#1	743279968	6.92E+08			
pfu/ml	6.40E+08			640436111				

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
Lewis M. Clark	[redacted]	Recorded by Sandrina Phipps	[redacted]

Project No. 414
TITLE RTPCR for Human Endostatin Book No. 1003

From Page No. [redacted]

Plated HeLa S cells 3 plates to set up transfection efficiency for pAmBg1X plasmid. pass 32

47 } 35.66x10^4 cells/ml Need 7.2x10^6 cells / 35.66x10^4 cells/ml
30 } Need 4x10^5 cells/4ml/well. = 21ml
30 } Used all of 20ml. There will be slightly less than 4x10^5 cells on each well.

20ml + 55ml media = plate 4ml/well.

Received oligos for Human & Canine Endostatin as of day June 8, 1998

For human endostatin PCR of Human Liver Poly A RNA we'll we need to use primers 2190 - sense, 2188 & 2189 antisense

2190 → 2.3mg/ml - mwt - 5793.8 g/mole

2.3 g/liter / 5793.8 g/mole = .00040 x 1000 mMoles/moles = 0.4000 x 1000 uMoles/mMoles = 400uM

Made 1uM dilution 1.25 ul / 500ul

2188 2.6 g/liter / 7046.6 g/mole = .00037 x 1000 mMoles/moles = 0.37 x 1000 uMoles/mMoles = 370uM

Made 1uM dilution 1.35ul/500ul

2189 1.7g/liter / 6787.4 g/mole = .0001 moles/liter x 1000 mMoles/moles x 1000 uMoles/mMoles = 103uM

1uM = 5ul/500ul H_2O

4ul $MgCl_2$ (25mM)
2ul 10x PCR Buffer II
2ul H_2O
8ul dNTPs (10mM)
1ul RNASE Inhibitor
1ul MuLV Reverse Transcriptase
1ul Oligo d(T)16
1ul human Liver RNA (100ng/ul)
1ul PFU (2.5U/ul)

Human Liver Poly A+ RNA - purified guanidinium thiocyanate → purified 2X oligo (dT)-cellulose columns
Lot # 6120365 Clontech (1ug/ul)
5ug total received.

10min @ room temp
15min @ 42°C
5min @ 99°C
5min @ 5°C
4°C Hold Cycle

To Page No. 126

Witnessed & Understood by me, Date [redacted]
Invented by
Recorded by
Date [redacted]

Project No. 414
Book No. 1003 TITLE PCR of Human Endostatin

From Page No. 125

[redacted] RTPCR After 4°C hold EtoH precipitated 2ul 3M NaAOC → 400ul EtoH. Store @ -70°C for 15min. Centrifuged 30min @ 4°C Washed in 70% EtoH. Resuspended pellet in 74.5ul H_2O

Setup PCR rxn with PFU

1X Buffer	10ul	(10X PFU Buffer)		95°C 3min
1uM primer	1ul	(1uM)	2190	Hold @ 80°C for 3min
1uM primer	1ul	(1uM)	2188 2189	35 cycles 1min @ 95°C
1.5mM MgCl2	6ul	(25mM)		2min @ 60°C
0.7mM dNTPs	7.5ul	(10mM)		2min 20sec @ 72°C
dH2O	74.5 ~~68.5~~ ul			Extend 10min
PFU enzyme	1ul	(2.5u/ul)		Hold @ 4°C
	100ul			gel picture page 127

PCR (88/90) → 2.0Kb (2189/90 → 1.790Kb)

Setup 10ml culture glycerol of SQ11 from Qiang Kang to make Calcium prep.

Sandra Phipps [redacted]

[redacted] Set up Transfection Efficiency of Hep 3B cells with Lipofectamine plus reagent

	DNA		Plus Reagent	Optimem	Lipofectamine	Optimem	Make 3 RXNs
A	3ul (1ug/ul)	pAvinBglx	18ul	300ul	6ul	300ul	total amt on plate 269ul
B	3ul	"	18	"	12ul	300ul	211ul
D	6ul	"	30	"	15ul	"	217ul
E	6ul	"	31ul	"	30ul	"	222ul
F	3ul	"	24ul	"	15ul	"	214ul
G	6ul	"	—	"	15ul	"	207ul

1. Incubate DNA plus reagent serum free media 15min RT
2. Mix lipofectamine & serum free media to pre complex 15min RT
3. Wash cells 1X with optimem media (1ml)
4. Add 0.8ml of optimem media overlay the precomplex / lipofectamine reagent ~200ul.
5. Incubate 1 set of 6 well plates 3 1/2 hr. remove transfection + add 4ml EMEM + 10% FBS. 1 set of plates left transfection mix on O/N, then I will change media

To Page No. 128

Witnessed & Understood by me, Yoan M Chuhe Date [redacted] Invented by Date [redacted]
Recorded by Sandra Phipps

128

Project No. 414
Book No. 1003 TITLE RT PCR Human Endostatin / Plasmid Prep

From Page No. 127 [redacted] SQ1

Processed Bulk plasmid prep of pSQ1 - as described on page 61.
Processed to Overlay ... Cesium gradient Spinning ON @ 55K.

Setup new RT-PCR reaction for human endostatin since no amplification products were visible the original time.
I will use random hexamer & downstream primer 2188 this time for priming (instead of oligo dT)

4ul MgCl2 (25mM) — 10min @ room temp
2ul 10x PCR buffer — 15min @ 42°C
2ul dep H2O — 5min @ 99°C
8ul dNTPs (1mM) — 5min @ 5°C
1ul RNase Inhibitor — Hold @ 4°C
1ul MuLV reverse transcriptase
1ul Random Hexamer or 2188
1ul RNA (1ug) - human liver poly A+

20ul rxn.

EtOH ppt 2ul NaOAc + 300ul EtOH ppt → Resuspend 20 ul ...

Setup PCR (cDNA) — 3min @ 95°C
20ul cDNA — 80°C 3min. - Add PFU (2.5u) 1ul
10ul 10x PFU Buffer — 35 Cycles 1min @ 95°C
1ul 2190 — 2min @ 58°C
1ul 2189 — ~~2min @ ...~~ 2min 20sec 72°C
7.5ul dNTPs — Extend 15min @ 72°C
59.5ul H2O — Hold @ 4°C ON
1.0ul PFU Hot Start
Poured 1% Seaplaque gel to run products ON.
Did a quick look ... No
From pg 126
Added 4ml of fresh EMEM + 10% FBS to transfected ... 3B cells
Which were left ON ≈ 24 Hr post transfection in Lipofectamine.

To Page No.

Witnessed & Understood by me, Date [redacted] Invented by Date [redacted]
Recorded by

From Page N [redacted]

PROTOCOLS FOR REVERSE TRANSCRIPTION OF RNA AND PCR AMPLIFICATION OF cDNA

Book 1003 page 129 35 [redacted]

PERKIN ELMER

PERKIN ELMER

To Page No.

Witnessed & Understood by me, Date [redacted]

Invented by Date [redacted]

Recorded by

TITLE RT PCR of Human Endostatin. Project No. 414 Book No. 1003 133

From Page No. [redacted]

- I will use (PAW109) RNA control Perkin Elmer Kit. Refer to protocol on page 129
- I will use random hexamer & downstream primer for PAW109 RNA control DM152
and 2188 for human endostatin. I will also use Taq + PFU Enzyme.

25 mM $MgCl_2$	4ul	✓	
10 mM dNTPs	8ul	✓	
10 PCR II Buffer	2ul	✓	
H_2O	2ul	✓	
RNase inhibitor	1ul	✓	
Random Hexamer	1ul		@ 1ul 20um 2188 or 1ul DM152 ✓
RT	1ul	✓	
	20ul rxn.		

Perkin Elmer RT-PCR program
25min@42°C
5min@99°C
5min@5°C
Hold@4°C

- I accidentally started Hot Start program instead of RT-PCR program
- I realized in time, however tubes had briefly warmed to 95°C
- I added fresh RT enzyme.

After RT precipitated PFU rxns because need to change buffs.
Set up Tag mastermix

78ul - mastermix for Endostatin RNA

4ul $MgCl_2$ (25mM)		
8ul PCR Buffer 2		
45.5ul H_2O	65.5ul H_2O	
10ul 2190 (1um) primer conc.	or 1ul DM152	
10ul 2189 (1um) " "	1ul DM151	
100ul		

Add 0.5 ul Sample Hot Start @ 80°C

↓ PAW109 control RNA

3min@95°C
3min@80°C - Add Taq
35 Cycles 1min@95°C
1min@60°C
1min 30sec@72°C
Extend 10min@72°C
Hold@4°C

When I programmed machine @ cycle 4 I left on hold @ 60°C
The rxns set @ 60°C for 2½ hrs before starting cycles as described above.
Lori Clark froze samples @ -20°C [redacted]

I went to set up PFU PCR - I added perkin Elmer buffer II instead of PFU Buffer. I decided to do the weekend.

To Page No. 134

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by [redacted] Date [redacted]

Recorded by [redacted]

Project No. 414
Book No. 1003
TITLE RT PCR / Preparation for Homologous Recombination of AVEI1X2 + AvnBgl2 Virus (50)

From Page No. 133 [redacted]

I EtOH ppt the RT PCR rxns from page 133. Setup PFU PCR rxns.

20ul cDNA (H2O) for Oncostatin RNA
10ul PCR Buffer (PFU)
10ul 2190
10ul 2189
7.5ul dNTPS (10mM)
39.5ul H2O
1ul PFU @ Hot Start (2.5u/ul)
1 drop/rxn overlay mineral oil

PAW109
1ul DM152
1ul DM151
39.5ul H2O

I accidentally added 10ul of prim 2189 + 2190 also.

3min @ 95°C
3min @ 80°C - Add PFU
35 Cycles 1min @ 95°C
1min @ 60°C
3min @ 72°C
Hold @ 72°C for 10min.
Hold @ 4°C. ON

Setup digests of pAVEI1X2 to use in transfections on Hep3B cells for Homologous recombination Studies. Need to linearize plasmid.

6ug (1.6ug/ul) 3.75ul
5.0ul 10XBSA
5.0ul Buffer 3
3.0ul NotI
50.5ul H2O.

Incubate @ 37°C ON.

Plated 8 6well plates of Hep3B cells @ ≈ 4.0x10^5 cells/well for transfections of Homologous recombination Story.

49
57 } 55.7x10^4 cells/ml Need 21x10^7 cells 1x10^5 cells/ml x 210ml =
61
Added media up to 210ml. I probably do not have exactly 4.0x10^5/well, but it's very close.
Plated 1 T150 1:20 passage 34

To Page No.

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

TITLE Continuation of Homologous Recombination of — Project No. 414 — Book No. 1003

From Page No. 142 [redacted] pAVE1LX2 + SQ1Virus

I split passage 32 S8 cells plated 1x10⁴ cells/well in 96 well plate to do TCID50 titer of SQ1-P2 virus from Vicky North which I used in Homologous recombination study.

173
109 } 137.6x10⁴ cells/ml — Need 5x10⁴ cells/ml x 22ml = 1.1x10⁶ cells / 137.6x10⁴ cells/ml
131

= 0.8ml + 21.2ml media + 6.6ul 100uM dexamethasone

Plated 200ul of cells/well. 1 96 well plate.

[redacted] Sandrina Phipps

[redacted]

I harvested 24 Hr time pt for homologous recombination study of SQ1-P2/pAVE1LX2 as described on page 142. Snap froze pellets on Sat. June 20th collect 72Hr time pt.

I added 6x10⁹ particles/ml to 96 well S8 plate to look for virus production this is to confirm that the SQP2 virus used for homologous recombination study is active.

Put 10ul of 6x10⁹ particles/ml /top well. Diluted down the plate 1:10. Left bottom row blank. go to page 145

Stained the pAVnBglx transfection control for homologous recombination transfection efficiency.

Washed 2X wells PBS. Fixed with glutaraldehyde/formaldehyde solution 5min @ room temp. Followed protocol page 58 Book 893.

Set up RT rxn for human ind. stabilizer

		PE4	
	25mM MgCl₂	4ul	10min @ room temp
	10mM dNTPs	8ul	15min @ 42°C
	10X PCR Buffer	2ul	5min @ 99°C
	H₂O	2ul	5min @ 5°C
	RNase Inhibitor	1ul	Hold @ 4°C
RNA: PAW109 1ul, 100ng human Liver polyA RNA	Random Hexamer 2188 OM152	1ul	
	RT	1ul	

To Page No. 144

Witnessed & Understood by me, Lim M Clarke — Date [redacted] — Invented by — Recorded by Sandrina Phipps — Date [redacted]

Project No. 414
Book No. 1003 TITLE RT-PCR Endostatin

From Page No. 143 [redacted]

Ethanol precipitate RT-rxn added 2ul 3M Na Acetate + 40ul cold EtOH
Precipitate @ -70°C for 15min → Centrifuge 30min @ 4°C
Wash 1X 70% EtOH Set up PCR RXNS The DM152 primer PAD109 control tube
busted - will have no PCR rxn.

20ul cDNA ✓
10ul PCR Buffer Clontech PFU ✓ — 3min @ 95°C
8ul dNTPS — 3min @ 80°C Add PFU
10ul 2190 ✓ For PAD109 control use 1ul DM152 ✓ — 35 cycles 1min @ 95°C
10ul 2189 ✓ — 1ul DM151 ✓ — 1min @ 60°C
39ul H_2O ✓ — 57ul H_2O ✓ — 3min @ 72°C
1ul PFU Hot Start (2.5u/ul) — Extend 15min @ 72
100ul Rxn — Hold @ 4°C

Gel picture 145

Plated 293 Cells → passage 11 to set up 2 6 well plates for transfection of pAdEla(DL×)

99
68 } 79.7×10^4 cells/ml — need 1×10^5 cells/ml × 4ml/well = 4×10^5 cells/well
72 } — 1.0×10^5 cells/ml × 55ml = 5.5×10^6 cells / 79.7×10^4 cells/ml

Need 6.9ml of cells + 48.1ml Richter's + 10% FBS

Digest SQ1 cesium DNA
30ug = 40ul (0.76ug/ul) — Incubate @ 65°C ON
5ul Buffer 4
2.5ul BstB1 - Same as SfuI
2.5ul H_2O
50ul

To Page No.

Witnessed & Understood by me, Date [redacted]
Invented by
Recorded by Date [redacted]

Project No. [illegible]
Book No. [illegible] TITLE [illegible]

From Page No.
[redacted]

Setup reaction [illegible]

4ul 25mM MgCl2	[illegible]
3ul [illegible] dNTPs	15min @ 42°C
2ul RT buffer [illegible]	5 min @ 99°C
2ul H2O	4°C
1ul RNase Inhibitor	
1ul Random Hexamers	
± 2188 (RNA)	
1ul RT	
1ul . RNA (poly A [illegible])	
20ul	

Etoh precipitated 2ul 3M NaAc + 350ul EtoH

Setup PCR Reaction

39ul H2O + DEPC [illegible]	3min @ 95°C
10ul [illegible] Buffer (PFU)	3min @ 80°C - Add PFU
10ul 2190 [illegible]	35 cycles 1min @ 95°C
10ul 2189 [illegible]	1min @ 55°C
30ul dNTPS (2.5mM each)	3min @ 72°C
98ul - [illegible] 2ul (2.5u/ul PFU)	Extend 15min @ 72°C
	Hold @ 4°C ON

[illegible] amplification [illegible] from pg 163. It appears the [illegible] + SO4 → may have contamination [illegible] the [illegible] Will [illegible] it and [illegible] supernatant

Make 2 T75 [illegible] SF cells + [illegible] 0.3um passage 37 to amplify pNEB [illegible]
Plated 1×10⁶ cells/T75 → 1.15ml of 1.3×10⁶ cells/ml. [illegible] had already trypsinized & counted cells.
Plated 1 [illegible] plate 1×10⁴ cells/ml × 40ml = 4×10⁵ cells / 1.3×10⁶ cells
Need 0.308 ml

Plated 4ml/well 4×10⁵ cells/well

[illegible] broke out new vial of SF cells passage 17 into T75 flask frozen on 11/7/88. SSP

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	[redacted]	Recorded by [signature]	[redacted]

178

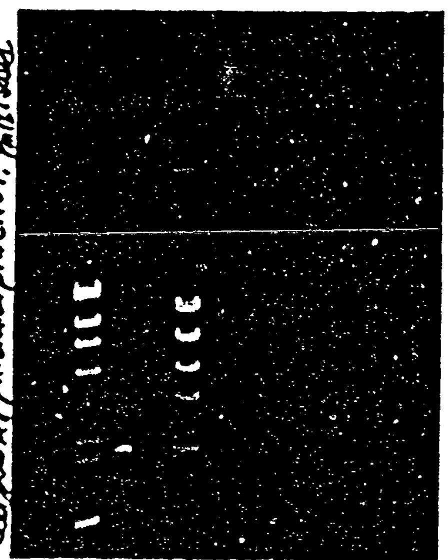
Project No. 414

Book No. 1003 TITLE Cloning of pAVE1a09LXi and Endostatin PCR-Script

From Page No. [redacted]

Set up ligation for pAVEILX2/pAVE1a09: to make pAVE1a09LXi.
Checked check gel to look @ gel purified fragments.

Gel purified pAVEILX2/pAVE1a09: PmlI/SalI



Book 1003 Pg 178 SSP [redacted]

Lanes 1 mw λHindIII/φX HaeIII
2 10ul pAVEILX2 PmlI/SalI
3 10ul pAVE1a09: PmlI/SalI
4 High mass ladder 4ul
5-10 Blk

7ul pAVEILX2 PmlI/SalI 6.3kb
11ul pAVE1a09: PmlI/SalI 3.6kb
2ul ligation Buffer
1ul ligase
21ul Incubate @ 15°C O/N.

Setup PCR-Script Cloning of Endostatin
Cl made 20ul rxns instead of 10ul reactions as in protocol because my fragment ...
1ul PCR-Script Amp SK(+) (10ng/ul)
2ul 1x Reaction Buffer
~~0.5ul~~ 1.0ul 10mM rATP
2.0ul SrfI
2ul T4 DNA ligase
12ul Endostatin human PCR product 790bp
20ul

Incubate @ room temp 25°C for 1Hr.
Heat @ 65°C 10min.
Place on ice until do ligation

Go to page 180

To Page No. 180

Witnessed & Understood by me. [signature] Date [redacted]

Invented by / Recorded by [signature] Date [redacted]

Project No. 414
Book No. 1003
TITLE Ligation + Transformation Protocol for PCR-Script - Strata

From Page No. [redacted]

I picked 15 colonies from PCR cut Ligation+transformation done on [redacted] protocol below. 3rd culture grew @ 37°C O.N.

Polishing the Purified PCR Products

Polish the ends of purified PCR products generated with native *Taq* DNA polymerase or other low-fidelity DNA polymerases as indicated in the following protocol.

Note *PCR DNA polymerase-generated PCR products do not require polishing. Proceed directly to Inserting the PCR Products into the pPCR-Script Amp SK(+) Cloning Vector.* Cloned used PFU

1. To prepare the polishing reaction, add the following components *in order* to a 0.5-ml microcentrifuge tube:

   10 µl of the purified PCR product
   1 µl of 10 mM dNTP mix (2.5 mM each)
   1.5 µl of 10× polishing buffer
   1 µl of cloned *Pfu* DNA polymerase (0.5 U)

2. Mix the polishing reaction gently and add a 20-µl mineral oil overlay.
3. Incubate the polishing reaction for 30 minutes at 72°C.
4. Add an aliquot of the polished PCR product directly to the ligation reaction (*see Inserting the PCR Products into the pPCR-Script Amp SK(+) Cloning Vector*) or store the polished PCR products at 4°C until ready for further use.

Inserting the PCR Products into the pPCR-Script Cam SK(+) Cloning Vector

Calculating the Insert-to-Vector Molar Ratio

This kit requires a high insert-to-vector molar ratio for ligation, higher than the molar ratios used in many other cloning procedures. The ideal molar ratio of insert-to-vector DNA is variable. The ligation in this kit is optimized to use an ideal test insert-to-vector ratio of 100:1. For the sample DNA, a range from 40:1 to 100:1 insert-to-vector ratio is recommended. Use the following equation to optimize conditions for the insert:

$$X \text{ ng of PCR product} = \frac{(\text{number of base pairs of PCR product}) \times (10 \text{ ng of pPCR-Script cloning vector})}{2961 \text{ bp of pPCR-Script cloning vector}}$$

where X is the quantity of PCR product (in nanograms) required for a 1:1 insert-to-vector molar ratio. The following table provides examples of optimal insert-to-vector molar ratios calculated using the above equation:

Size of PCR product (bp)	Quantity of PCR product (ng), 40:1–100:1
250	35–88
500	68–169
750	100–250
1000	130–330
1500	200–500
2000	265–660
3000	400–990

Book 1003 pg 180 SSP [redacted]

Ligating the Insert

1. To prepare the ligation reaction, add the following components in order to a 0.5-ml microcentrifuge tube:

   1 µl of the pPCR-Script Amp SK(+) cloning vector (10 ng/µl)
   1 µl of PCR-Script 10× reaction buffer
   0.5 µl of 10 mM rATP
   2–4 µl of the blunt-ended PCR product or 4 µl of the control PCR insert
   1 µl of *Sma*I restriction enzyme (5 U/µl)
   1 µl of T4 DNA ligase
   Distilled water (dH_2O) to a final volume of 10 µl

2. Mix the ligation reaction gently and incubate this reaction for 1 hour at room temperature.
3. Heat the ligation reaction for 10 minutes at 65°C.
4. Store the ligation reaction on ice until ready to use to transform the Epicurian Coli® XL1-Blue MRF' Kan supercompetent cells.

TRANSFORMATION GUIDELINES

Supercompetent Cells

Epicurian Coli XL1-Blue MRF' Kan supercompetent cells are very sensitive to small variations in temperature and must be stored at the bottom of a –80°C freezer. Transferring tubes from one freezer to another may result in a loss of efficiency. Store the Epicurian Coli XL1-Blue MRF' Kan supercompetent cells on ice at all times while aliquoting. It is essential that the Falcon 2059 polypropylene tubes are placed on ice before the supercompetent cells are thawed and that the supercompetent cells are aliquoted directly into the prechilled polypropylene tubes. Pipet the remaining supercompetent cells into 40-µl aliquots and freeze the aliquots at –80°C. Do not pass the frozen supercompetent cells through more than one freeze-thaw cycle.

Note *The efficiency of the Epicurian Coli XL1-Blue MRF' Kan supercompetent cells should be ≥5 × 10⁸ cfu/µg. (Approximately 500 colonies are expected when 1 µl of supercompetent cells are plated.)*

Use of Falcon 2059 Polypropylene Tubes

It is important to use Falcon 2059 polypropylene tubes for the *Transformation Protocol*, because other tubes may be degraded by β-mercaptoethanol. Additionally, the critical incubation period during heat pulsing is calculated for the thickness and shape (i.e., round bottom) of the Falcon 2059 polypropylene tube.

Use of β-Mercaptoethanol

β-Mercaptoethanol increases transformation efficiencies two- to threefold. This kit includes prealiquoted, ready-to-use β-mercaptoethanol.

Length of the Heat-Pulse

Optimal transformation efficiencies are observed when transformation reactions are heat-pulsed for 45–50 seconds. Stratagene recommends heat-pulsing for at least 45 seconds to allow for variability in the duration of heat-pulse. Transformation efficiencies decrease sharply when heat-pulsed for <45 seconds or for >60 seconds.

Book 1003 pg 180 SSP [redacted]

T Page No. 1003

Witnessed & Understood by me, [redacted]
Date [redacted]
Invented by
Date [redacted]
Recorded by

TITLE Transformation Protocol (for PCR-Script - Stratagene Project No. 414 Book No. 1003

From Page No. 180 [redacted]

TRANSFORMATION PROTOCOL

[redacted] SSP Book 1003 (p.181)

1. Thaw the Epicurian Coli XL1-Blue MRF' Kan supercompetent cells on ice.
2. Gently mix the Epicurian Coli XL1-Blue MRF' Kan supercompetent cells. Aliquot 40 µl of the supercompetent cells into a prechilled 15-ml Falcon 2059 polypropylene tube for the experimental transformation reaction. For transformation of the ligation reaction containing the control PCR insert, aliquot 40 µl of the supercompetent cells into a prechilled Falcon 2059 tube. For a control transformation using the pUC18 control plasmid, aliquot 20 µl of the supercompetent cells into a prechilled Falcon 2059 tube.
3. To prepare the experimental transformation reaction and the transformation containing the control PCR insert, add 0.7 µl of β-mercaptoethanol to the polypropylene tubes containing the supercompetent cells to yield a final concentration of 25 mM. For the transformation reaction with the pUC18 control plasmid, dilute 1 µl of the provided 1.44 M β-mercaptoethanol with 1 µl of dH_2O, and add 0.7 µl of the diluted β-mercaptoethanol to the appropriate tube of supercompetent cells.
4. Swirl the transformation reactions gently. Incubate the transformation reactions on ice for 10 minutes, swirling the reactions gently every 2 minutes.
5. Add 2 µl of the experimental ligation reaction from step 4 of *Ligating the Insert* to the experimental transformation reaction and swirl the reaction gently. Add 2 µl of the ligation reaction containing the control PCR insert from step 4 of *Ligating the Insert* to the appropriate transformation reaction and swirl gently. Add 1 µl (0.1 ng) of the pUC18 control plasmid to the appropriate transformation reaction and swirl gently.
6. Incubate the transformation reactions on ice for 30 minutes.

   Note *Prepare the SOC medium and equilibrate the medium to 42°C.*
7. Heat-pulse the transformation reactions in a 42°C water bath for 45 seconds. The duration of the heat-pulse is critical for optimal transformation efficiencies (see *Length of the Heat-Pulse*).
8. Incubate the transformation reactions on ice for 2 minutes.
9. Add 0.45 ml of the prepared SOC medium to each transformation reaction and incubate the reactions at 37°C for 1 hour with shaking at 225–250 rpm.
10. Prepare the plates for color selection by spreading 20 µl of 0.2 M IPTG and 20 µl of 10% (w/v) X-gal on all agar plates 30 minutes prior to plating the transformation reactions.

    Note *Avoid mixing the IPTG and the X-gal before spreading them on the agar plates because these chemicals precipitate when combined. Prepare X-gal in dimethylformamide (DMF). Prepare IPTG in sterile dH_2O.*
11. Plate the experimental transformation reaction, the transformation containing the control PCR insert, and the pUC18 control transformation as follows:
    a. Use a sterile spreader to plate 200 µl (or less) of the experimental transformation reaction onto LB–ampicillin agar plates.[§]

       Note *The cells may be concentrated by centrifuging at 1000 rpm for 10 minutes if desired. Resuspend the pellet in 200 µl of NZY+ broth and plate.*

    b. Use a sterile spreader to plate 50 µl of the transformation reaction containing the control PCR insert onto a LB–ampicillin agar plate and 50 µl of the transformation reaction containing the control PCR insert onto a LB–chloramphenicol (30 µg/ml) agar plate.[§]

       Note *The control PCR insert is a Pfu DNA polymerase-generated PCR product that contains a chloramphenicol-resistance gene. The control transformation reaction should be plated on LB-ampicillin agar plates and on LB–chloramphenicol agar plates to verify that the transformed colonies are also chloramphenicol-resistant.*

    c. Use a sterile spreader to plate 5 µl of the transformation reaction containing the pUC18 control plasmid into a 200-µl pool of NZY+ broth on an LB–ampicillin agar plate.
12. Incubate the plates overnight at 37°C.
13. Choose white colonies for examination (avoid colonies with a light blue appearance or colonies with a blue center). If necessary, patch white colonies onto a new LB–ampicillin agar plate containing X-gal and IPTG to verify the *Lac⁻* phenotype.

    Note *Colonies containing inserts which were initially pure white may turn a light blue after ~2–5 days on the plate.*

Book 1003 pg. 181
SSP [redacted]

Transformation Summary and Expected Results

Control transformation	Quantity of transformation	Plating quantity	Expected results: LB–ampicillin agar plates	Expected results: LB–chloramphenicol agar plates
pUC18 control plasmid	1 µl	5 µl	500 colonies ($\geq 5 \times 10^8$ cfu/µg DNA)	—
Control PCR insert	2 µl	50 µl	>100 colonies	>100 colonies*

* Growth of colonies on LB-chloramphenicol plates indicates successful insertion of the control PCR insert, which contains the chloramphenicol-resistance gene.

PCR Script Cloning Map page 182

Setup transformation
4ul ligation rxn for [illegible] PCR followed protocol steps 1→10.

I plated 200+10ul on IPTG/X gal plates. Lori Clarke had stored my plates @4C on [redacted]

To Page No. 182

Witnessed & Understood by me, Lori M. Clarke [redacted]

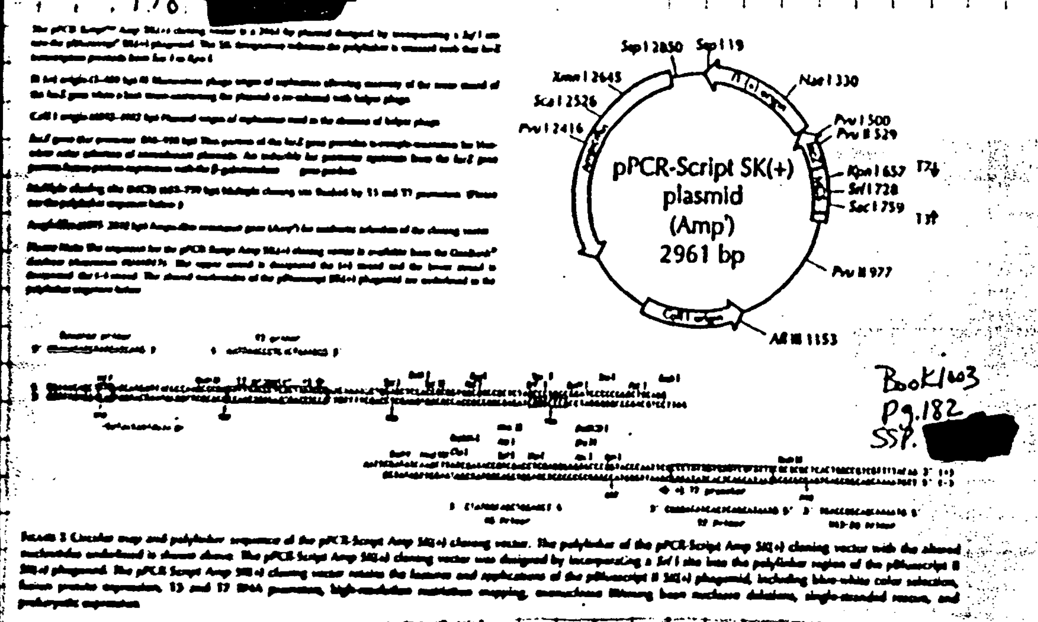
Invented by

Recorded by Sabine Phipps

Date [redacted]

Project No. 414
Book No. 1003 TITLE pPCR-Script SK(+) Stratagene Map

From Page No. 181 [redacted]
178



Book 1003
Pg. 182
SSP. [redacted]

From page 178
John Oakubczak had transformed my ligation of pAVEhd9 & pAVELY2
[redacted] while I was on vacation in XBL2 cells page 178
I picked colonies [redacted] 18 colonies - 3ml cultures Grew @ 30°C O/N

Go to page 183

I examined my TCID 50s on Hep 3B + 293 cells of Homologous Recombination Experiment page 177.
This is day 5 post infection

	Hep3B	293
SQ1-CVL	No rows show CPE	2 (Top 2 rows show CPE)
SQ1-P2 10^5 PFU/ml	Top Row CPE	6 rows CPE
SQ1+pAVELY2	2 rows CPE	7 rows CPE
SQ1 pAVELY2+ pCMV	4 rows CPE	7 rows CPE.

I will officially read plates 10 days post infection

I submitted pBeloBac plasmid stabs to core to be bulked by Qiagen prep
This was plasmid Paul Hallenbeck got from Human Genome Systems

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

Project No. 414
Book No. 1060 TITLE Restriction Digests pAVEla09Lxi / Maxiprep of PCR-Script human endostatin

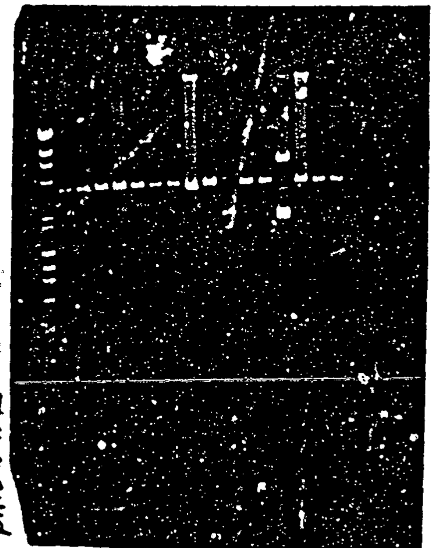
From Page No. 1 [redacted]

Ran check digests of pAVEla09Lxi on 1% agarose gel

Lanes

1	mut λ HindIII ΦX Hae III	15	(15) BsrGI/BamHI
2	miniprep #1 BsrGI/BamHI	16	16
3	2	17	17
4	3	18	18
5	4	19	Bk
6	5	20	Bk
7	6		
8	7		
9	9		
10	10		
11	11		
12	12		
13	13		
14	14		

Clone #14 is correct banding pattern 2.017, 1.87 & 6.052 Kb.



Book 1060 pg. 4 [redacted]

Qiagen maxiprep of PCR-script human endostatin clone 2 page 2
Resuspended 250ul H2O Took OD reading 1:100 dilution

~~[illegible]~~ [redacted]

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: Auto smplr
Read average time: 0.50 sec

Units: ug/ul
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [N]

Book 1060 page 4 SSP. [redacted]

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ul	Nucleic Acid ug/ul
1	0.0005	0.0002	2.3478	0.4259	0.0000	0.0013
2	1.4997	1.5216	0.9856	1.0146	0.0000	3.7493

1.4997 x 50ug/ml x 100 = 7.5mg/ml

Set up check digests for endostatin

1ul DNA	1ul DNA	1ul DNA
2ul Buffer 1	2ul Buffer 2	2ul Buffer 1
2ul 10x BSA	0.75ul HindIII	1ul XmaI
0.75ul SacI	0.75ul STuI	16ul H2O
0.75ul KpnI	15.5ul H2O	20ul
13.5ul H2O		

Incubate 37°C ON

To Page No.

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

TITLE Setup Limiting Dilution of SQ1-P2 To purify Away

Project No. 414

Book No. 1060

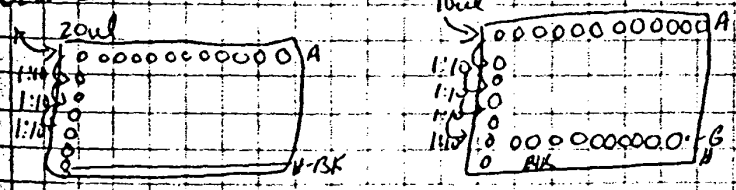
7

From Page No. 6 [redacted] from Wildtype Adenovirus

Set up transformation of pCR-Script human cerebellum clone 2
Diluted miniprep DNA #2 → 1:100. Used 1ul in transformation.
1ul clone 2 + 10ul PH.5x cells.
30min on ice 42°C-45sec - 2min on ice.
Added 90ul SOC shake/bake @ 37°C 1hr
Plated 15ul + 40ul LB plates + 0.1ug/ml Ampicillin
Also restreaked the clone 2 liquid cultures. Grew O/N @ 37°C

Setup limiting dilutions for cleaning up SQ1-P2 virus (AvnBglx virus) away from wildtype adenovirus. Plated 3T6 cells on [redacted] page 5
Need 10 particles/cell in each well of 96 well plate.
SQ1-P2 (TCA55T) virus was 1.92×10^{12} particles/ml
10 particles/cell $\times$ 2×10^4 cells = 2×10^5 particles
2×10^5 particles/well $\times$ $\frac{1 \text{ well}}{0.02 \text{ ml}}$ = 1×10^7 particles/ml Needed for dilution
1×10^7 particles/ml $\times$ 1ml = 1×10^7 particles
Made 2 (1:100 dilutions) and then took 52ul into 1ml → 1:100
Made 1:100 dilution 52ul virus / 5000ul media (1.92x10^10 particles/ml) 10ul / 1000ul (1.92x10^8 particles/ml)
Took 52ul / 1000ul
for 1×10^7 particles.

Plated 20ul into top row of 96 well plate made 1:10 dilutions down through row G.
Plated 10ul into top row of 96 well plate made 1:10 dilutions down through row G



The dilutions will titrate down to 1 particle/1 cells.

To Page No.

Witnessed & Understood by me, Lisa M Clarke Date [redacted]

Invented by

Recorded by Barbara Wapp Date [redacted]

Project No. 414
Book No. 1060 TITLE Closing Bulk Prep pAVEla 09Lxi, Midiprep PCR-Script Junction Identification/Ratio of

From Page No. [redacted]

Took temperature plate of clone 2 PCR-Script Junction Identification page 7 Southern Picked one colony grew up in LB + 0.1 g/ml ampicillin @ 37°C

Prepared bulk cesium prep on pAVEla09Lxi clone 14 via procedure page 33 Book 823 Thru cesium banding

Made 3 dpcm/stocks of pAVEla09Lxi clone 14 (2 ml cultures + 2 ml 50% glycerol) Store @ -70°C

Hybridized Southern blot BstnI/BglII from Book 1003 page 159, 161, 179
Used gap DNA probe from Loi Clarke.

Prehybridized blot - hybridization buffer + 100 ul Salmon Sperm DNA/ml boiled for 5 min. Hybridized approx 4 hr.
Added 15 uCi of probe 68 ul of 0.22 uCi/ul. Probed O/N @ 68°C.

Calculated band ratios of Southern Blot BstnI/BglII. This was probed from Book 1003 p. 179 with DNA which would identify plasmid, input virus, and new recombined virus AVE1LX2. The probe on page 149 Book 1003 was a 2.5 Kb fragment EcoRI/XhoI pAVNBglX

Need to analyze 1 more gel + average, however it appears that the original SQ1-virus band is about 3.6-fold higher than the AVE1LX2 virus. The newly recombined virus

I will reanalyze blots tomorrow [redacted]

-1021

Book 1060 page 8 SSP [redacted]

	Volume		
RECT- 1	4871.52	3518	- SQ1 virus
RECT- 2	2311.41	- 957.78	AVE1LX2 virus
RECT- 3	1353.63	- Background	

} 3.7 fold difference between SQ1 & AVE1LX2 virus

To Page No. 9

Witnessed & Understood by me, Lisa M Clarke Date [redacted]

Invented by

Record d by Dorothea Yapp Date [redacted]

TITLE BM40 Signal Peptide for Human Endostatin

Project No. 414
Book No. 1060

From Page No. [redacted]

Ordered BM40 signal peptide oligos to add onto human endostatin PCR product. Michele Kaloss said that once oligos go above 100 bases that the fidelity of adding bases onto oligo may be altered, thereby giving wrong sequence. I went ahead and made 118 & 125-mer oligo. I will sequence after cloning.

Oligo Request Form

Name: Sandrina Phipps	Project Code: 414	Date: [redacted]
Length of Oligo: 118		
Purification: ☐ Unpurified ☒ OPC (oligo purification cartridge)	Note: oligos ≥ 40 bases will be OPC purified	
Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)		

Sequence written 5' to 3' in triplets:

HindIII / BM40 signal

GCC [AAG CTT] CCA TCA GGG CCT GGA TCT TCT

TTC TCC TTT GCC TGG CCG GGA GGG CTC TGG

CAG CCC CTC AGC AAG AAG CGC TCG CTC ACA (human endostatin)

GCC ACC GCG ACT TCC AGC CGG TGC TCC A

Total #G 32 #A 18 #T 24 #C 44

Book 1060
pg. 37 SSP [redacted]

Purpose: Cloning of BM 40 signal oligo into human endostatin plasmid. Approx 100 bases of BM40 signal peptide + human endostatin sequence 117 to 147 of pcrhend1 plasmid. Sense oligo has SexA1 restriction site at 3'end.

Name: Sandrina Phipps	Project Code: 414	Date: [redacted]
Length of Oligo: 123		
Purification: ☐ Unpurified ☒ OPC (oligo purification cartridge)	Note: oligos ≥ 40 bases will be OPC purified	
Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)		

Sequence written 5' to 3' in triplets:

CCA GGT GGA GCA CCG GCT GGA AGT CGC GGT

GGC TGT GAG CGA GCG CTT CTT GCT GAG GGG

CTG CCA GAG CCC TCC CGG CCA GGC AAA GGA

GAA AGA AGA TCC AGG CCC TCA TGG AAG CTT GGC

Total #G 46 #A 25 #T 18 #C 34

Book 1060
pg. 37 SSP [redacted]

Purpose: Clone the BM40 signal peptide onto human endostatin PCR product pcrhend1. The BM signal peptide is approx 100 bases followed by human endostatin sequence 154 to 116 of pcrhend1 seq. Antisense oligo.

```
Molecule Name:      pcrhend1              3750 bps DNA Circular
Sequence Printed:   1-3750 (Full)         Date Printed [redacted]
Description:        Ligation of Fragment 1 and pCR-ScrptSK+

  1  cagatgacat cctggccagc ccccctcgcc tgcccgagcc ccagccctac
      Q  M  T  S  W  P  A  F  L  A  C  P  S  P  S  P
       R  -  H  P  G  Q  P  P  S  P  A  R  A  P  A  L
        D  D  I  L  A  S  P  P  R  L  P  E  P  Q  P  Y

 51  cccggagccc cgcaccacag ctcctacgtg cacctgcggc cggcacgacc
     T  P  E  P  R  T  T  A  P  T  C  T  C  G  R  H  D
      P  R  S  P  A  P  Q  L  L  R  A  P  A  A  G  T  T
       P  G  A  P  H  H  S  S  Y  V  H  L  R  P  A  R

101  cacaagccca cccgcccaca gccaccgcga cttccagccg gtgcagcacc
      P  Q  A  H  P  P  T  A  T  A  T  S  S  R  C  S  T
       H  K  P  T  R  P  Q  P  P  R  L  P  A  G  A  P
     P  T  S  P  P  A  H  S  H  R  D  F  Q  P  V  L  H

151  tggttgcgct caacagcccc ctgtcaggcg gcatgcgggg catccgcggg
      W  L  R  S  T  A  P  C  Q  A  A  C  G  A  S  A
     P  G  C  A  Q  Q  P  P  V  R  R  H  A  G  H  P  R
      L  V  A  L  N  S  P  L  S  G  G  M  R  G  I  R  G

201  gccgacttcc agtgcttcca gcaggcgcgg gccgtggggc tggcgggcac
     G  P  T  S  S  A  S  S  R  R  G  P  W  G  W  R  A
      G  R  L  P  V  L  P  A  G  A  G  R  G  A  G  G  H
       A  D  F  Q  C  F  Q  Q  A  R  A  V  G  L  A  G
```

human endostatin → primer SexAI

Book 1060
Page 37
SSP [redacted]

The endostatin sequence highlighted in pink was added to BM40 signal peptide. The oligo goes through SexA1 site.

To Page No.

Witnessed & Understood by me,	Date [redacted]	Invented by	Date [redacted]
Lori M. Clark		Recorded by Sandrina Phipps	

Project No. 414
Book No. 1060 TITLE RT-PCR Canine Endostatin

From Page No. [redacted]

Diluted Canine Endostatin oligos to do RT PCR primer sequences are on Book 1003 pages 130-131 Sequence on page 115

				Dilution 20uM / 500ul
2230 - 3.9mg/ml	3900 / 0.33 x 22-mer	= 3900 / 7.26	= 537uM	18.6ul oligo / 500ul
2231 - 3.1mg/ml 21-mer	3100 / 6.93	= 447uM		22.37ul oligo / 500ul
2232 4.2mg/ml 24-mer	4200 / 792	= 530uM		18.9ul oligo / 500ul

4ul 25mM MgCl2
8ul 10mM dNTPs
2ul PCR Buffer II
2.5ul H_2O
1ul RNAse Inhibitor
1ul Random Hexamer or Oligo dT or 2231
0.5ul Canine RNA (22ug total 4.4ug/ul)
1.0ul RT (murine Maloney)

10 min @ room temp
15 min @ 42°C
5 min @ 99°C
Hold 4°C

EtoH ppt 2ul 3M NaOAc + 300ul EtoH Freeze -70°C 15 min.
Centrifuge 30 min. Wash w 70% EtoH.
Resuspend DNA 57ul H_2O Set up PCR with PFU enzyme

57ul DNA
0.5ul 2231 (10uM final)
0.5ul 2232 (10uM final)
10ul PFU Buffer 10X
30ul dNTPs (2.5mM each)
98ul

3 min @ 95°C
3 min @ 80°C Add PFU
35 Cycles 1 min @ 95°C
1 min @ 58°C
3 min @ 72°C
Extend 15 min @ 72°C
Hold @ 4°C

Hot Start add 2ul a ~~1 unit~~ 5 units of PFU
SSP

Showed Theresa migration assay slides & discussed procedure

To Page No. 39

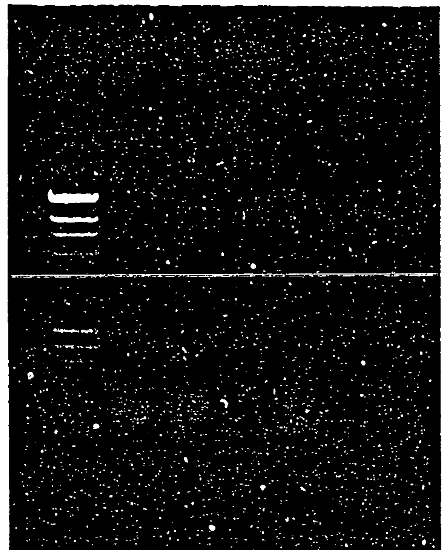
Witnessed & Understood by me, [signature] Date [redacted] Invented by Date [redacted]
Recorded by [signature]

TITLE Canine RT PCR / Cloning of Human Endostatin with   Project No. 414   Book No. 1060   39

From Page No. 38 [redacted]   BM40 Signal Peptide

Ran endostatin PCR for Canine on gel
Lanes
1 mut λHindIII φXHaeIII
2 oligo DT - 2231/2232 90ul
3 Random Hexamer - 2231/2232 90ul
4 Bk
5 2231 - 2231/2232 90ul
6 Bk

I cut out bands between 600+800bp region, will try to reamplify this


Canine RT PCR
Book 1060 SSP [redacted] page 39

Page 42 (has)
Cloning scheme for BM40 Signal & human endostatin
Digested pCRhend1 - SexA1 - because cuts in different buffer from HindIII.
This will be used to clone BM40 oligos onto human endostatin PCR product
(Clone 2)

5ul pCRhend1 (Clone 2) [redacted]
238ug/ul
2ul BSA 10X
2ul Buffer 3
1.5ul SexA1 (4u/ul)
9.5ul H2O
20ul

Incubate @ 37°C t_0 = 11:00
t_f = 1:00
EtOH ppt digest 2ul 3M NaOAc + 300ul EtOH
Put @ -70C 30min. Centrifuged resuspended in 15ul H_2O

go to page 40

I received BM40 signal peptide oligos Sequence page 37
Annealed oligos together so I can cut with HindIII to clone into HindIII/SexAI site of pCRhend1

5.45ul oligo 2732 (6ug) 1.1ug/ul
4.6ul oligo 2733 (6ug) 1.3ug/ul
1.5ul Buffer 2
3.45ul H_2O
15ul

Incubate PCR machine under DASS program
Refer to Book ~~1063~~ SSP 893 page 19

To Page No. 42, 40

Witnessed & Understood by me, [signature]   Date [redacted]   Invented by   Recorded by [signature]   Date [redacted]

Project No. 414
Book No. 1060 TITLE Cloning of BM40 Signal Peptide onto Human Endostatin

From Page No 39 [redacted] PCR

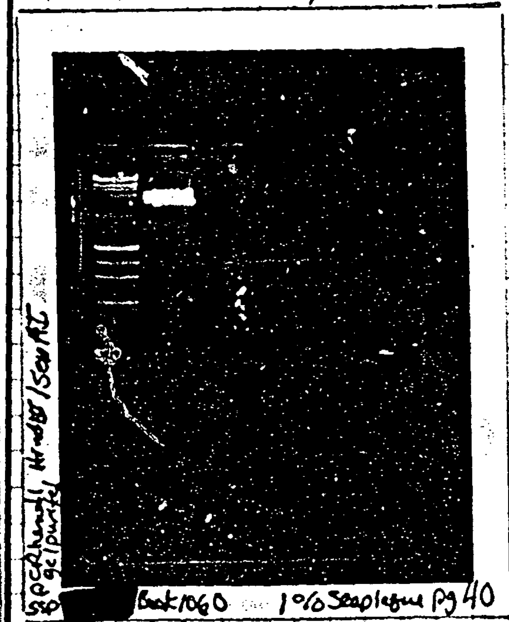
pCRhend 1
15ul H_2O/DNA
2ul Buffer 2
1.5ul HindIII (20u/ul)
1.5ul H_2O
20ul

Oligo 2732
2733
15ul Oligo DNA
0.5ul Buffer 2
1.5ul HindIII
3ul H_2O

Incubate @ 37°C 2H.

Gelpurify pCRhend1 (HindIII/SexA1) to isolate 3.6Kb fragment.

Passed oligo through millipore micro enzyme extremial column. Washed 5ul of elution. EtOH ppt. Resuspended 10ul. (0.6ug/ul)



Book 1060 1% Seaplaque Pg 40

Isolated band marked with arrow. Did Promega IC DNA cleanup Kit.

Setup ligation
4.25ul oligo (annealed ≈ 2.55ug SexA1/HindIII
8.0ul PCRscript hend 1 SexA1/HindIII
2.0ul Ligase buffer
4.75ul H_2O
20ul Incubated @ 15° ON.

Need to run check gel to make sure Gelpurified fragment is clean.

Collected (SSP)
I collected AVE1a OSLxi virus CVL amplified 9 days on 293 cells upon original 3 plasmid transfection. I did not see CPE.

I obtained Hep 3B cells transfected with CSIRo#100 conjugated by Ke Wang with X-gal. Followed procedure Book 893 page 58. Left X-gal on ON. Ke Wang did luciferase assay. I was staining for blue cell #

To Page No.

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

From Page No. [redacted]

Applied Biosystems

Synthesis Order - [redacted]

Customer Name:
[redacted]

Customer Address:

Book 1060
page 41
SSP [redacted]

Telephone Number: Fax Number:
PO Reference: Account Number:
Entry date: [redacted]
Comments:

1.3 mg/ml

Run Protocol: SynPur 4.20
Post Synthesis: Purify Oligo
Sequence Name: 2733.898
Sequence:
5' CCA GGT GTA GCA GCG GCT GGA AGT CGC AGT GGC TGT GAG GCA GCG GTT CTT GCT GAG GGG CTG
CCA GAG CCC TCC CGG CCA GGC AAA GGA GAA AGA ACA TCC AGG GTT TCA TGG AAG CTT GGC 3'
Sequence Length: 101
Base Composition:
A:25 G:046 C:036 T:0[illegible]

Request form for these oligos on page 37 and partial sequence of endostatin used.

Applied Biosystems

9:03:23 AM [redacted] 1948Control 2.2

Synthesis Order - 2732.898

Customer Name:
[redacted]

Customer Address:

Book 1060
page 41
SSP [redacted]

Telephone Number: Fax Number:
PO Reference: Account Number:
Entry date: [redacted]
Comments:

1.1 mg/ml

Run Protocol: SynPur 4.20
Post Synthesis: Purify Oligo
Sequence Name: 2732.899
Sequence:
5' GGC AAG CTT CCA TGA GGG CCT GGA TCT TCT TTC TCC TTT GGC TGG CCG GGA GGG CTC TGG CAG
CCC CTC AGC AAG AAG CGC TCG CTC ACA GCC ACC GCG ACT TCC AGC CGG TAC TCC A 3'
Sequence Length: 118
Base Composition:
A:018 G:332 C:044 T:024 5:030 6:030 7:000 8:010

Pooled 158 cells to amplify AVE1a09Lxi virus and re-plated 14 T150 58 cells → 1:20 to make roller bottles next well.
Passage 20

110 } 99.4 x 10^4 cells/ml Need 1.33 x 10^5 cells/ml x 24ml = 3.2 x 10^6 cells / 99.4 x 10^4 cells/ml
89 }

Need 32ml + 20.8ml of Media + 72ul dexamethasone (100um) 0.3 final
Need 4 x 10^5 cells/well
Plated 1 6 well plate 3ml/well.

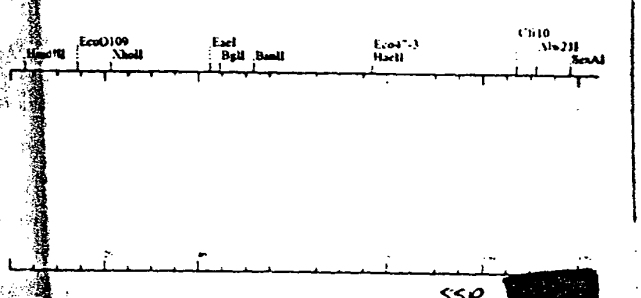
To Page No.

Witnessed & Understood by me, [redacted] Date [redacted]
Invented by [redacted] Date [redacted]
Recorded by [redacted]

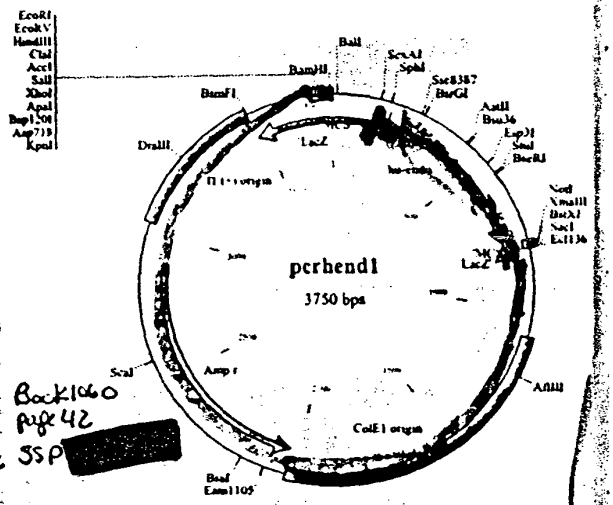
Project No. 414
Book No. 1060 TITLE Cloning Scheme of BM40 Signal Peptide Addition

From Page No. 29 [redacted] to Human Endostatin PCR product.

We made BM40 signal peptide oligo with SexA1 overhang as it had already been cut.



SSP [redacted]
Book 1060 pge 42



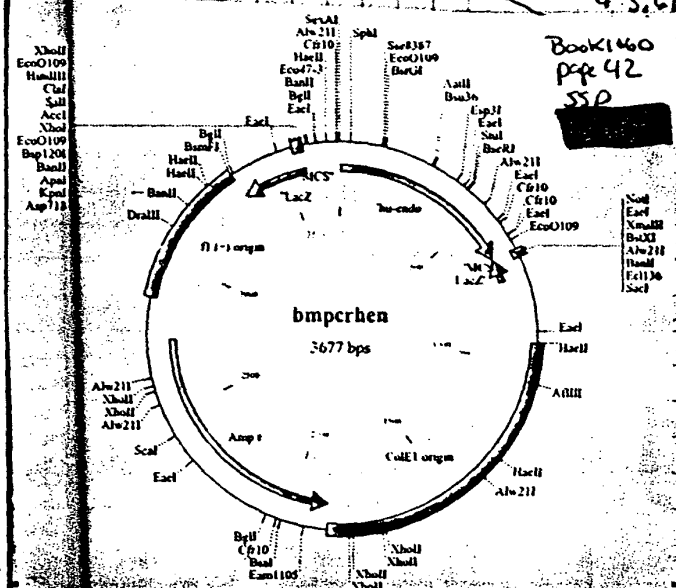
Book 1060
pge 42
SSP [redacted]

Cut pcrhend1 (HindIII)/SexA1)
Cut BM40signalpeptide HindIII/Hco SexA1site
HindIII

114bp

HindIII/SexA1
Isolate 3.6Kb fragment

Ligate 114 bp
& 3.6Kb fragment



Book 1060
pge 42
SSP [redacted]

Sequence of BM40 signal peptide is on page 41

To Page No.

Witnessed & Understood by me, [redacted] Date [redacted] Invented by [redacted] Date [redacted]

Recorded by [redacted]

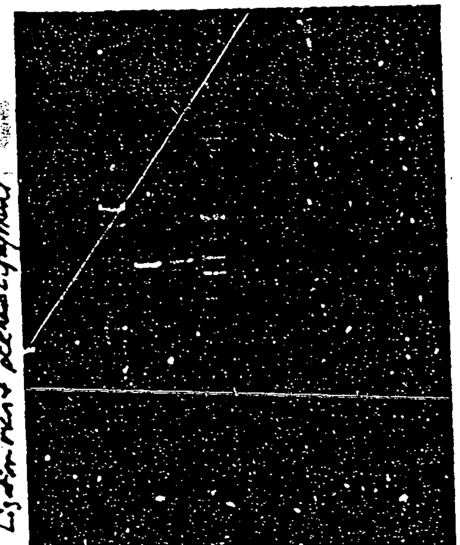
TITLE Transformation of BM40 Signal peptide + Human Endostatin PCR. Project No. 414 Book No. 1060

From Page No. — [redacted]

Ran check gel to look @ ligation rxn of BM40signal + human endostatin (bm pcr hen)
Ran gel purified pcRhend I (HindIII/SexAI) fragment 3.6Kb.

Lanes
1. mw + λ HindIII φ X HaeIII
2. 3.6Kb gel purified pcRhend I HindIII/SexAI 8ul
3. ligation rxn bm pcr hen 7ul
4. high mass marker 4ul
5-10 - Blank SSP

It appears ligation occurred + pcRhend I fragment contains rxn.
Freeze thawed AVE1a 09Lxi CVL 5X to infect S8 cells / dexamethasone.



Ligation rxn + pcRhend I fragment

Book 1060 pg 43 [redacted] SSP

Set up transformation
4ul ligation rxn pcRhend I / BM40signal + 100ul STBL2 cells
30 min on Ice
Heat shock 25sec @ 42°C
2min Ice
Add 900 ul to ligation rxn incubate @ 30°C 1½ hr.
Plated 100 + 200ul on LB plates + ampicillin.
Grew ON @ 30°C.

Collected T75 CVLs of AVE1a 10 09Lxi SSP virus in S8 cells from page 34 SQ1-P2 R5-10, 11, 3, 7. I threw out R5-8 because it looked contaminated. Collected SQ1-P2 R6-6 + 6-9. I threw away R6-8 because it looked contaminated. I began amplifying the AVE1a09Lxi virus CVL which had been grown with 3 plasmids in 293 cells on 6 well plates. From page 41.

Plated 0.5ml of CVL AVE1a09Lxi onto 4 wells of 6 well dish. Added 1.5 ul of 100uM dexamethasone to each well. Put @ 37°C 5% CO2

To Page No. ___

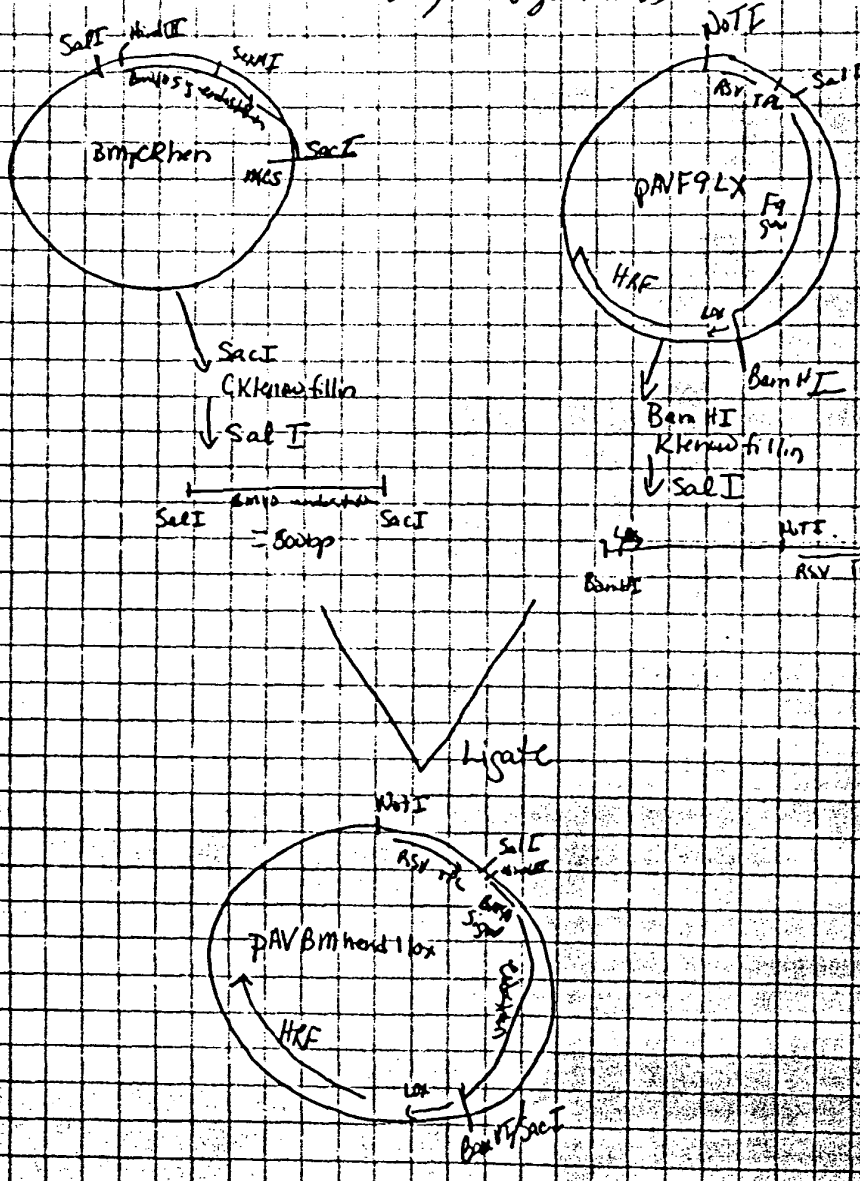
Witnessed & Understood by me,	Date	Invented by	Date
[signature]	[redacted]	Recorded by [signature]	[redacted]

Project No. 414
Book No. 1060
TITLE Cloning Scheme for Human Endostatin with

From Page No. [redacted]

BM40 Signal peptide attached with Lox site

Set up digests. In BM40pcRhanI and F9LX plasmid. I am showing it sequenced; however I will redo and show ready if sequencing correct



Page No. 65

Witness d & Understood by me, [redacted]

Date [redacted]

Invented by

Recorded by

Date [redacted]

TITLE Cloning of pAVB0 hand I Lox / OD Readings for SQ1P2

From Page No. 64 [redacted]

Viral genomic DNA

Digest B.mpkhand I Sac I
16ul 5ug 0.305ug/ul
2.5ul Buffer 1
2.5ul BSA 10x
1.5ul Sac I
2.5ul H2O
25ul Incubate O/N @ 37°C

pAVF9Lx Bam HI
5.7ul (10ug) 1.74ug/ul
2 ul BamHI Buffer
2ul BSA 10x
1ul BamHI
9.3ul H2O
20ul

[redacted]

[redacted]

1.0ul Klenow
25ul BmpkhandI SacI
0.5ul Buffer 1
1.8ul 1mM dNTPs
2.7ul H2O
30ul

20ul pAVF9Lx BamHI
1.8ul 1mM dNTPs
1.0ul Buffer BamHI
1.0ul Klenow
6.2ul H2O
30ul SSP

Incubate @ 25°C 15 min. - actually went approx 45 min Heat inactivated 15 min @ 75°C EtOH precipitated go to page 66

OD Readings of SQ1-P2 genomic Viral DNAs

Book 1060 page 65
SSP [redacted]

Sample	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ul	Nucleic Acid ug/ul
	-0.0001	0.0001	-0.7965	-1.2555	0.0000	-0.0002
	-0.0001	0.0002	-0.3981	-2.5117	0.0000	-0.0602
	0.0001	-0.0001	-0.7963	-1.2553	0.0000	0.0002
RS-7	0.0523	0.0374	1.3323	0.7179	0.0000	0.1301
	0.0527	0.0379	1.3919	0.7185	0.0000	0.1317
	0.0523	0.0377	1.4015	0.7135	0.0000	0.1319
RS-11	0.0441	0.0229	1.9267	0.5190	0.0000	0.1103
	0.0446	0.0228	1.9562	0.5112	0.0000	0.1115
	0.0449	0.0232	1.9375	0.5161	0.0000	0.1123
RS-9	0.0216	0.0081	2.5864	0.3722	0.0000	0.0541
	0.0227	0.0087	2.6081	0.3834	0.0000	0.0568
	0.0232	0.0094	2.4748	0.4041	0.0000	0.0580
RS-3	0.0399	0.0162	2.4684	0.4051	0.0000	0.0998
	0.0403	0.0165	2.4439	0.4082	0.0000	0.1008
	0.0405	0.0166	2.4454	0.4059	0.0000	0.1012
	0.0001	-0.0004	-0.1992	-5.0212	0.0000	0.0002

.0525 x 50ug/ml x 50 = 131.25ug/ml = .131ug/ul
.0445 x 50 x 50 ug/ml = 111.25ug/ml = 0.111ug/ul
0.0225 x 50 x 50ug/ml = 56 ug/ml = .056ug/ul
.0402 x 50ug/ml x 50 = 100ug/ml = .1ug/ml

To Page No. 66

Witnessed & Understood by me, Lori M Clarke | Date [redacted] | Invented by | Recorded by Sandra Phipps | Date [redacted]

Project No. 414
Book N . 1060
TITLE Cloning of BM40pCRhen into pAIF9LX / Canine Endosta

From Page No. 65

[redacted]

PCR

Setup digests with SalI for pAVF9LX & pBM pCRhen I which have been cut with SacI, BamHI and Klenow filled in.

2ul SacI Buffer
2ul 10X BSA
1ul SalI
15ul DNA (Resuspended pellet 15ul H_2O)
20ul

Incubate ON @ 37°C

from page 63
Phenol/chloroform/isoamyl alcohol extraction … viral genome … , chloroform extracted, EtOH precipitated
Resuspended in 75ul H_2O.

Setup PCR nested with PCR rxns shown on page 57

10ul PCR rxn 2231/2230 ,, 2231/2232
2ul MgCl2
5ul Buffer 2 PCR
0.5ul 2231
0.5ul 2230
1.0ul 10mM dNTPs
0.5ul Amplitaq Gold
30.5ul H_2O
50ul

12 min @ 94°C
35 cycles 1min @ 95°C
1 min @ 56°C
1 min @ 72°C
Hold 10min @ 72°C
Hold @ 4°C ON.

Split 293 cells passage 28 + 13 T150 1:20

[redacted]

Sandrine Phipps

[redacted]

Ran 2% Seaplaque gel for PCR product 2231/2230

Lanes
1 mwt λHindIII ΦXHaeIII
2 2231/30 pcR - using 2231/30 primer 5ul
3 2231/32 pcR " " "
4 Low mass DNA marker 4ul
5-6 BLK

Ran 1% Seaplaque gel for DNA isolation

Lanes
1 mwt λHindIII ΦXHaeIII
2 pAVF9LX BamHI Klenow / SalI
3 BM40 pCRhen SacI Klenow / SalI
4 low mass DNA marker

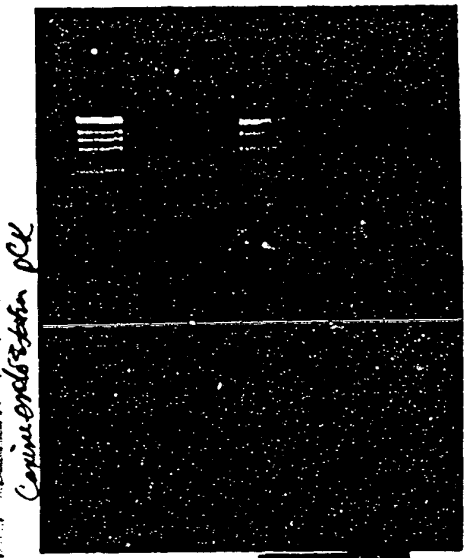
To Page No. 67

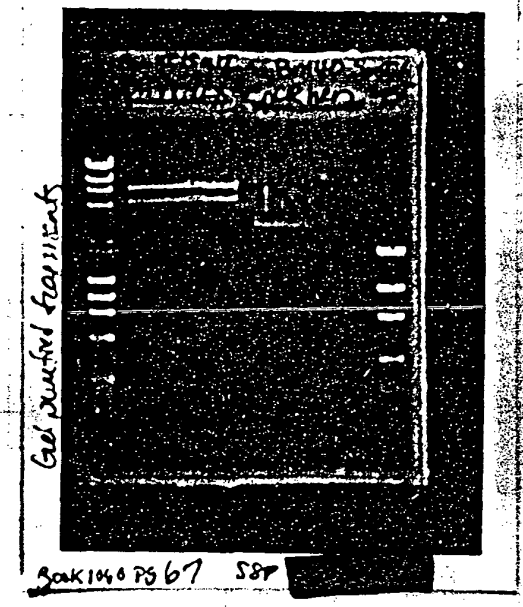
Witnessed & Understood by me, [signature] Date [redacted]

Invented by
Recorded by [signature]
Date [redacted]

From Page No. 66

[redacted] PCR





Cloned arrowed fragment from pAVF9Lx+ BM40pCRhen digested DNA.
The Canine PCR did not work.
Cloning to isolate poly A mRNA from dog liver.
Gel purified 6.9 & 0.800 kb gel fragments Promega PCR Clean up kit.
Ran 10ul on check gel on next page 68. The DNA is too dilute to see, however; I set up ligation anyway to transform

2ul ligase buffer
8ul pAVF9Lx (6.9 BamHI/SalI)
10ul BM4 pCRhen (0.8 Kb SacI/SalI)
1ul ligase
21 ul
Incubate ON @ 15°C

To Page No. 68

Witnessed & Understood by me, [redacted] Date [redacted] Invented by [redacted] Date [redacted]
Recorded by [redacted]

Project No. 414
Book No. 1060
TITLE TCID50 Titer SQ1-P2, AVEh10Lxi, Production of SQ1-P2 R5-7

From Page No. 92 [redacted]

From page 92

Processed the SQ1-P2 R5-7 virus CVL from 8 roller bottle prep. as described pg. 20 Book 1003. Did 1.25/1.4 mg/ml CsCl-SW28 spin and ON spin in NVT65 @ 60,000K in 1.33 mg/ml CsCl

Processed Hep 3B cells - trypsinized + washed + put into 1% formaldehyde in PBS for ... negative controls for ... Split Hep 3B 1:10 passage 52

Split 1 plate of S8 cells for transfection of AV3 + AV hu human endostatin virus. I forgot to linearize plasmids so therefore, I needed to plate new cells. Gave S8's plated on [redacted] for Lori Clarke. (Passage 17)

136] 132×10⁴ cells/ml   I need 4×10⁵ cells/ml × 30 ml = 1.2×10⁷ cells
128]   / 132×10⁴ cells/ml
= Need 2.3 ml + 90 ul ...

Linearized SQ3, SQ4 plasmid + pBMhsd^L X for 3+4 5 way transfection

SQ4 (4.3 ug/ul) 3.5 ul
15 ug   2.0 ul Buffer H
H₂O   12.5 ul
2.0 ul Cla I 10 u/ul
20 ul

SQ3 (2 ug/ul) 10 ul
20 ug   2.0 ul Buffer H
6.0 ul H₂O
2.0 ul Cla I 10 u/ul
20 ul

pBMhsd^L X (3.1 ug/ul) 9.68 ul
30 ug   2 ul Buffer
2 ul BSA
4.32 ul H₂O
2.0 ul Not I
20 ul

Incubate @ 37°C ON

Read TCID50 for titer SQ1-P2 R5-3 + AVEh10Lxi - 1 roller bottle preps

Book 1060
pg. 94
SSP [redacted]

> 3.45×10⁹ pfu/ml
20 ul/well

Sample: S8 cells SQ1-P2 R5-3

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	+	+	+	+	+	+	+	+	+	+	+
B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
D	+	+	+	+	+	+	+	+	+	+	+	+
E	+	+	+	+	+	+	+	+	+	+	+	+
F	+	+	+	+	+	+	+	+	+	+	+	+
G	+	+	+	−	−	−	+	−	[illegible]	+	+	+
H												

Dilution: 1:100 dilution from 10⁻¹ particles/ml to 10⁻⁹ particles/ml

Sample: AVEh10Lxi Virus [redacted] S8 cell 1:100 dilution
20 ul/well

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E	+	+	+	+	+	+	+	−	+	+	+	+
F	+	−	−	−	−	−	−	−	−	+	−	−
G	−	−	−	−	−	−	−	−	−	−	−	−
H												

Sept. 29, 1998
Book 1060
pg. 94
SSP
1.23×10⁸ pfu/ml

To Page No. 95

Witnessed & Understood by me, Lori M Clarke   Date [redacted]
Invented by
Recorded by Sandra ... Phipps   Date [redacted]

From Pag No. 94 [redacted] Plasmid System

Pulled virus band of SQ1-PZR5-7 total volume 2.8ml added 2.8ml/9ml = 0.311 ml of glycerol. Dialyzed in cassette through 4 changes of HBS Buffer 1x + glycerol @ 4°C - 4 liters. Last dialysis went O/N.

Set up 3 plasmid transfections of SQ3 + SQ4 with pBMhindIx plasmid

pSQ3 (ClaI)	1ug	1ul	} +6ul plus reagent +10ul Optimem media
pCre	0.3ug	1ul	
pBMhindIx (NotI)	0.5ug (1.5ug/ul)	0.5ul	

Set up 2 rxns → put on 58 cells.

pSQ4/ClaI	1ug	1.33ul (0.75ug/ul)	} +6ul plus reagent +100ul Optimem media
pCre	0.5ug	1ul (0.5ug/ul)	
pBMhindIx part 2	0.5ug	0.5ul (1.5ug/ul)	

Set up 2 rxns → put on clone 54 cells.
Incubate 15 min @ room temp.
Add 4ul lipofectamine +100ul Optimem. Made 4 1/2 rxns 45ul media + 18ul lipofectamine

Incubate @ room temp. for 15 min.
Add 0.8ml of Optimem media to cells overlay 200ul of DNA/lipofectamine complex to cells.
Incubated @ 37° 5% CO2 4 1/2 Hr. Removed transfection mix
Added 4ml of R. Ch Hes + 5% FBS to clones 54 + 58 cells.

Set up 5 way transfection to SQ4 to make AV4 virus on 293 cells in 35mm wells (6 well plate)

pC-Cre (Cre)	1ug	(2ul)	or	1ug	(2ul)
pGRES-E4 (E4)	0.5ug	(0.5ul)		0.5ug	0.5ul
pCE2a (E2a)	0.5ug	(0.5ul)		0.5ug	0.5ul
pSQ4cutw/ClaI	1.5ug	(2.0ul)		1.0ug	1.33ul
pBMhindIx/NotI - shuttle plasmid	3.75ug	(2.5ul)		2.5ug	1.67ul
Use Calcium phosphate kit	H_2O	102ul			103.5ul
	$CaCl_2$	15.5ul			15.5ul
		125ul			125ul

To Page No. 96

Witnessed & Understood by me. Lisa M Clark Date [redacted] Invented by [redacted] Recorded by [redacted]

Fr m Page No. 95

[redacted] Production

Bubbled DNA+Hg CaCl2 complex into 2x Hepes White Vortexing.
Incubate @ room temp 30 min.
Overlay 250ul onto 2 wells of 6 well plate
Changed media Richters + 10% FBS before dosing transfection - approx 3 Hr.
Incubated 37°C 5% CO2

Setup TCID50 titer plate for SQ1-P2 R5-3 because original TCID50 titered off the plate.

Took the 9/18 1:100 dilution Made another 1:10 dilution, then used this take to make 10-fold dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} input onto 96 well plate

10^{-1}	Row B	10^{-5} Row E	To Calculate TCID50
10^{-2}	Row B	10^{-6} Row F	the original starting dilution
10^{-3}	Row C	10^{-7} Row G	Will be 1:10,000.
10^{-4}	Row D	Blank control Row H	I put 200ul in each well

[redacted]

[redacted]

Aliquoted SQ1-P2 R5-7 SSP4-98 Virus
22 vials 20ul
20 vials 50ul
3 vials 100ul
Virus

Prepared particle concentrations
10ul virus incubate @ 56°C with shaking 10 min.
90ul viral concentration buffer incubate @ room temp 10 min.
Took 260nm OD. Made 1:10 dilutions 1 OD = 1.12×10^{12} particles/ml
1. 0.335 $0.335 \times 10 \times 1.12 \times 10^{12}$ particles/ml = 3.752×10^{12} particles/ml
2. 0.180 $0.180 \times 10 \times 1.12 \times 10^{12}$ particles/ml = 2.016×10^{12} particles/ml
3. 0.178 $0.178 \times 10 \times 1.12 \times 10^{12}$ particles/ml = 1.99×10^{12} particles/ml
I think I must have used 20ul in #1 tube it is approx double
Samples 2+3
Averaged # 2+3 together for particle titer = 2.025×10^{12} particles/ml
Made 1:100 dilutions of other 10ul to titer virus in Richter's + 5% FBS To Page No. 97

Witnessed & Understood by me, [redacted] Date [redacted]
Invented by [redacted] Date [redacted]
Recorded by [redacted]

TITLE AVE1a09Lxi particle titer / Amplification

Project No. 414
Book No. 1060

From Page No. [redacted] AV3 human endostatin Virus

Collected 2nd amplified CVL from AV3 + human endostatin on 58 cells 7 days post infection. Centrifuged & removed media to 3 ml. Resuspended CVL in 3 ml media.
This was 2nd amplification of SQ3 + human endostatin which was transfected in 88 cells with lipofectamine plus. Froze @ -70°C.

Lot #
Set up particle OD readings for AVE1a09Lxi SSP5-98
Took 20ul of 20ul aliquot vial
(2) 20ul out of 50ul aliquot vial

Added 80ul Viral concentration buffer to each tube (Book 1003 pg. 25)
Incubate @ 56°C 10 min with Shaking.
Incubate @ RT. 10 min. Read 260nm OD. Dilution made was 1:5

Read ODs in duplicate

BECKMAN DU-650 Date: [redacted] Time: 10:47

AVE1a09Lxi

Book 1060 page 119

S. Phipps [redacted]

Nucleic Acid
Read Samples | Method | Save Clear | Print | Quit

Results file: A:\NUCL_RES Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No]

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml	
1	0.0743	0.0655	1.1348	0.8812	0.0000	0.1857	0.076 x 5 x 1.12x10^12 particle/ml = 4.256 x 10^11 particles/ml
2	0.0774	0.0674	1.1484	0.8708	0.0000	0.1934	
3	0.0723	0.0637	1.1341	0.8818	0.0000	0.1806	0.073 x 5 x 1.12x10^12 particle/ml = 4.088 x 10^11 particles/ml
4	0.0734	0.0644	1.1402	0.8766	0.0000	0.1836	
5	0.0728	0.0632	1.1513	0.8682	0.0000	0.1820	0.073 x 5 x 1.12x10^12 particle/ml = 4.088 x 10^11 particles/ml
6	0.0741	0.0646	1.1462	0.8725	0.0000	0.1852	

50ul aliquot and 20ul
20ul
50ul aliquot

Average 4.14 x 10^11 particles/ml

Particles/ml 4.14 x 10^11 particles/ml
AVE1a09Lxi SSP5-98

To Page No.

Witnessed & Understood by me, Kim M Clarke
Date [redacted]
Invented by
Recorded by Sandra Phipps
Date [redacted]

From Page No. 120

[redacted] of Human Endostatin.

Freeze thawed Homologous Recombination Hep3B amplified CVLS 4X as well as AV4-3 plasmid clone 54. CVL from 2nd amplification of 3 plasmid transfection collected CVL 7 days post infection - freeze thawed 4.4X as well as AV3- human Endostatin 3rd Round amplification.

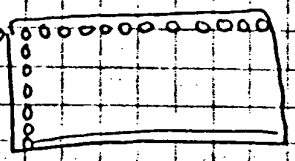
Put 2nd of AV3 human endostatin (2nd amplified CVL) from 3 plasmid transfected on 5 Bcells onto T75 1X10^7 Sf cells + dexamethasone 0.3uM this is 3rd amplification.

Collected AV4 - 2nd amplification CVL from 293 cell (5 plasmid transfection) which had been amplified on clone 54 cells.
Collected CVL/supt. 12mls for 1:67 ug + 375ug of DNA used.
Centrifuged - removed 9ml of supt stored @ 4°C for endostatin test and Resuspended 3ml Unto Cells. Store @ -70°C Combined 3 wells of each together in 50ml conical tube.

Set up TCID50's of SQ1-P2 R5:7 (20ul aliquot SSP4-98 Lot#
AVE1a09Lxi (20ul aliquot SSP5-98-Lot#

Diluted SQ1-P2 1:10,000 because original 1:100 titered off plates)
1:100 → 1:100 FMEM+5%FBS
10ul/990 → 10ul/990 → Made serial 10fold dilution 100ul/900 → 100ul/900
10^{-1} 10^{-4} 10^{-7} Put 20ul unto each well using combitip.
10^{-2} 10^{-5}
10^{-3} 10^{-6}

Diluted AVE1a09Lxi 1:1000 in FMEM+5%FBS
1:100 → 1:10
10ul → 990 → 100ul/900 Made serial dilution 10^{1} → 10^{-7} 100ul → 900ul



Set up viral genomic DNA extractions of SQ1-P2 R5-7+ AVE1a09Lxi virus
20ul Incubate @ 56C with shaking for approx 3-4H.
20ul TES Phenol Chloroform extract / Chloroform Isoamyl alcohol extract.
5.72ul proteinase K Ethanol ppt ON @ -20°C.
Vortex well

To Page No.

Witnessed & Understood by me, [redacted] Date [redacted] Invented by [redacted] Date [redacted]
Recorded by [redacted]

Project No. 414
Book N . 1060 TITLE Collection of Homologous Recombination CVLs / Collection of AV4 Human Endostatin CVLs

From Page No. 131 [redacted]

Plated 1 T150 1:3 + 75ul dexamethasone to amplify AV3 human endostatin
Plated 5 T150 @ 1:10 to set up for roller bottles for making AV3 human endostatin.

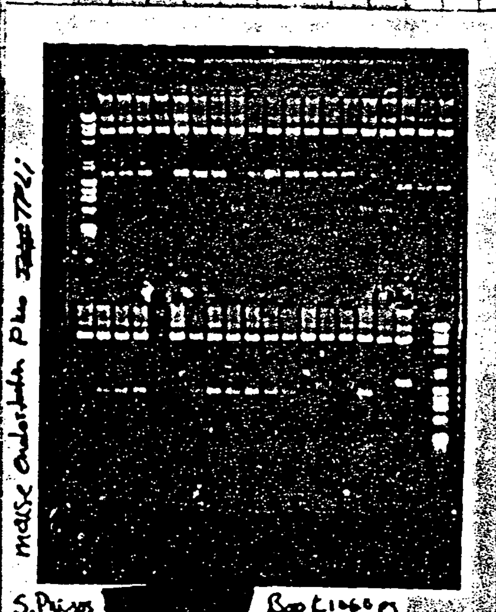
Collected CVLs of homologous recombination experiment page 126, 130.
I collected 1a,b, 5a,b, 6a,b, 7a,b, 8a,b, and SQ1-P2 alone after day 6 postinfection. I need to reamplify 5-8 on Hep 3B cells.
Snap froze dry ice, froze @ -70°C.

Collected AV4 clone 54 human endostatin 3rd amplification → 3 wells of 6 well plate CVL and supernatant. I collected cells + sup. Centrifuged 5min @ 1800RPM → removed 8ml of supt stored @ 4°C for human endostatin elisa testing. Froze remaining cells + supt for CVL @ -70°C

Collected AV4 human endostatin 3rd amplification made by 5 plasmid in 293 and passaged 2x on clone 54 cells. I had used 1.67 + 3.75ug of DNA. Collected 6 wells + pooled together for each DNA concentration. Centrifuged + removed 15ml of supt. Made CVL out of cells + remaining sup. Stored sup @ 4°C

Ran gel for pAVTPLi and LX clone gel
1% Agarose
Top gel
Lanes
1 - mw λ HindIII ΦX HaeIII
2 ~~1-17~~ 16 minipreps AscI/NheI
18-20 minipreps # 1-3 NotI

Bottom gel
Lanes
~~1~~ HP
1-4 minipreps # 4-7 NotI
5 - BK
6-16 minipreps # 8-18 NotI
17-18 - minipreps # 17-18 NheI/AscI
19 - BK
20 - mw λ HindIII ΦX HaeIII



S.Phipps [redacted] Book 1060 p.

To Page No. 133

Witnessed & Understood by me, Date [redacted] Invented by Date [redacted]
Recorded by Sandra Phipps

TITLE B... From Pag... Fro... Ce... Rea... Lo... Rep... Po... Tu... Met... Witnessed

Project N. 414
Book No. 1060 TITLE Bulk Preps of AVTPLi BMHendLx and Lx

From Page No. [redacted]

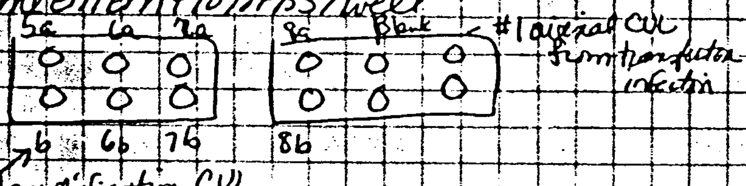
3rd Amplification of Homologous Recombination CVL

↓ clone 3 ↓ clone 1
Grew up 500 ml cultures of pAVTPLi BMhendLX and pAVTPLi endLX
in Yet Broth + PO4 + 100 μg/ml ampicillin.
Grew ON @ 30°C for Bulk plasmid preps

Freeze thawed AV3 humanendostatin 3rd amp CVL and Homologous Recombination supernatant CVLs from Hep3B cells 5x.

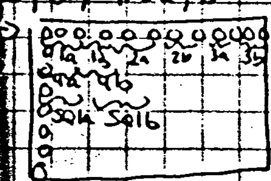
Put 3rd amplification of Homologous Recombination CVLs onto Hep3B cells plated on page 131.

Plated 5a, b, 6a, b, 7, a, b, 8a, b and #1 original pAVF9Lx 4μg + SQ1-virus onto 2 6well plates.
Incubated ON @ 37°C 5% CO2 1ml on each well rocked ON
At 20 hr post infection will remove CVL wash 2X PBS and add 4ml DMEM + 10% FBS/well



I should have used 1st amplification CVL for #1 after transfection infection 1a + b collected on 10/19/98 instead of original. I am reamplifying #1 because 2nd CVL has some CPE but it was next to wells highly infected with AVELX2 + SQ1 @ 4μg/well which had been harvested. We want to make sure this CPE was not caused from splash over or cross over from well to well while harvesting was done. t_0 = 12:30 pm

Setup 58 amplification of 2nd Round amplification of CVL from #1a, b, #2a, b #3a, b #4 a, b, SQ1 a+b



I did duplicate wells of each CVL for PCR 10ul/well. On Friday I will denature cells & prepare for PCR

To Page No. 137

Witnessed & Understood by me, Date [redacted] Invented by Date [redacted]
Lin M Clark
Recorded by Sandra Phipps

Project No. 414
Book No. 1060 TITLE Infection of Various Cell Lines With AVE[illegible]

From Page No. 149 [redacted]

AV Ea09Lxi

For HUVEC cells I only used 2X10^4 cells/well. I had new dilutions for infection

10 particles/cell X 2X10^4 cells/well X 1 well / 0.1ml = 2X10^6 particles/ml

2X10^6 particles/ml X 1ml = 2X10^6 particles - need 8.3ul Add1312 / 1ml
12.6ul Add1327 / 1ml
48.3ul AVEa09Lxi / 1ml
14ul AVEa10Lxi / 1ml

1. All well were infected in duplicate.
2. Washed wells 1X PBS.
3. Added 0.1ml virus onto each well except for LS180 which gets 0.2ml of virus.
4. Infect @ 37°C and 5% CO_2 with rocking for 3Hr
5. Washed wells 1X after removing virus then added ~~[illegible]~~ fresh media appropriate for the cells 2ml/well.

It appears part of wells may have dried out. I need to infect with 0.2ml if this experiment is repeat.

Met with Mike Perry 1 1/2 hr - all hands meeting

From page 146

I infected ~~w~~ three wells of AV 4 human endostatin 3rd round amplified CVL of 6 well plate 0.4ml / well. This was only remaining CVL after contamination. I filtered through 0.2uM filter. Reamplified 4th time Clone 54 cells. (2.5ug)

Infected 4 wells of clone 54 cells (3.75ug DNA) AV 4 human endostatin 3rd round amplified ~~CVL~~ ASP CVL.

Infected 1 well of clone 54 cells with AV4 human endostatin made 3 plasmid system in Clone 54 cells

Infected 8 roller bottles with AV3 human endostatin 30ml IMEM + 2% FBS + 0.3uM dexamethasone + 1ml of CVL / bottle

After 3Hr added IMEM + 5% FBS up to 150ml.

Incubate @ 37°C 5% CO_2

To Page No.

Witnessed & Understood by me, [signature] Date [redacted]

Invented by

Recorded by [signature] Date [redacted]

Project No. 414
Book No. 1060 TITLE X-gal Staining of Transient Transfection 293T cells of pAVHPL i and Lxd BMhandLx

From Page No. 155 [redacted]

I X-gal stained the 4 plates transfected with pAVnBgLX.

Washed plates 1x PBS. Added formaldehyde, glutaldehyde fixative 5ml (10min @ 4°C)
Washed plates 1X PBS and added X-gal reaction mixture.
Stained @ 37°C 5% CO2 for approx 4hr.
Staining procedure book 893 page 58.

Prepared AV3 BMhandLx (human endostatin) CsCl on prep - 8 roller bottles
Carried through to O/N spin in NVT65 Book 1005 pg. 19-21.

Split Chang cells P3 1:20 T_{150}
Split SaOS-2 cells - McCoy's + 15% FBS + P/S 1:8 in 3 T_{150} to freeze some cells down.

Split S8 cells p24 1:3 into 3 flasks.

Set up 20 293 96 well plates for TCID50 of Hand/genes/recombinant CVLS 1a - 8a, b.
293 passage 23
88 }
67 } 85x10⁴ cells/ml — Need 5x10⁴ cells/ml x 430ml = 2.15x10⁷ cells
100 } — Need 25.3 ml cells + 404.7 ml media
Plated 200ul/well → 1x10⁴ cells/well.

Plated a 6 well plate to set up transfection of AV4 human + mouse TP into endostatin again.

1x10⁵ cells/ml x 27ml = 2.7x10⁶ cells / 85x10⁴ cells/ml Need 3.2ml cells.
(Plated 20 96 well plates)
Split Hep 3B cells passage 60
44 }
38 } 41.33x10⁴ cells/ml — Used all cells to try to plate close to 1x10⁴ cells/well
42 } in 96 well plates. Plated 19 plates.
30ml of cells x 41.33x10⁴ cells/ml = 1.24x10⁷ cells / 380ml = 3.3x10⁴ cells/ml
Plated 200 ul of 3.3x10⁴ cells/ml for 6.5x10³ cells/well

To Page No. 157

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

TITLE Human Endostatin Elisa

Project No. 414
Book No. A60

From Pag No. 156 [redacted]

The 293 cells transfected on page 151 - many of cells rounded up & come off plate (AV4 human mouse TPL intron endostatin); therefore I may repeat transfection

Sandra [signature] [redacted]

[redacted]

Pulled cesium band virus AV3 human endostatin processed on page 156. Measured band 2.15ml added 0.239ml glycerol (1:(215/9ml)) Centrifuged, loaded in dialysis cassette Dialyzed in THP Buffer 4X - 4 liters total @ least 1hr / liter.
Freeze-thawed Homologous recombination cells 5X
Set up human endostatin elisa

Samples were as followed (Supernatant from AV3+AV4 human endostatin)

1. Human TPL intron endo + nBg } Transient Transfection Supt in 293 cells
2. human endo + n Bg [redacted] 72 hr post transfection supt
3. Human TPL intron endo
4. Human endo endo
5. mouse end
6. mouse TPL intron endo
7. mouse TPL intron n Bg
8. mouse endo n Bg
9. AV3 human endostatin (from 8 roller bottle prep [redacted]
10. AV3 human endostatin Supt 3rd amp [redacted]
11. AV4 human endostatin supt Clone 54 2nd amp [redacted]
12. AV4 clone 54 (293 5 plasmid) 3.75μg 3rd amp [redacted] 6 day p.i.
13. AV4 Clone 54 (293 5 plasmid 1.67μg 3rd amp [redacted] 6 day p.i.
14. AV4 clone 54 (Clone 54 3rd round amp) [redacted] 6 day p.i.
15. AV4 Clone 54 (293 5 plasmid) endostatin 2nd amp 1.67μg DNA [redacted] 7 day p.i.
16. AV4 Clone 54 (293 5 plasmid endostatin 2nd amp 3.75μg DNA [redacted] 7 day p.i.

Layout of endostatin plate on page 168
Followed standard elisa protocol 500ng/ml → 1.95 ng/ml
detection. Protocol pages 105-106

To Page No. 158

Witnessed & Understood by me, [signature]
Date [redacted]
Invented by
Recorded by Sandra [signature]
Date [redacted]

158

Project No. 414
Book N. 1060 TITLE Endostatin Elisa

TITLE

From Page N. 157 [redacted] Elisa Plate Format

Book 1060 page 158 S. Phipps [redacted]

	1	2	3	4	5	6	7	8	9	10
A	standard	1.95	#1 1:2	#5 1:2	#9 1:2	#13 1:2	#1 1:4	#5 1:4	#9 1:4	#13 1:4
B	standard	1.95	#1 1:2	#5 1:2	#9 1:2	#13 1:2	#1 1:4	#5 1:4	#9 1:4	#13 1:4
C	1.25	ODmx	#2 1:2	#6 1:2	#10 1:2	#14 1:2	#2 1:4	#6 1:4	#10 1:4	#14 1:4
D	1.25	ODmx	#2 1:2	#6 1:2	#10 1:2	#14 1:2	#2 1:4	#6 1:4	#10 1:4	#14 1:4
E	3.125 ~~7.81~~	1:2 mdx	#3 1:2	#7 1:2	#11 1:2	#15 1:2	#3 1:4	#7 1:4	#11 1:4	#15 1:4
F	3.125	1:2 mdx	#3 1:2	#7 1:2	#11 1:2	#15 1:2	#3 1:4	#7 1:4	#11 1:4	#15 1:4
G	7.81	1:4 mdx	#9 1:2	#8 1:2	#12 1:2	#16 1:2	#4 1:4	#8 1:4	#12 1:4	#16 1:4
H	7.81	1:4 mdx	#11 1:2	#8 1:2	#12 1:2	#16 1:2	#4 1:4	#8 1:4	#12 1:4	#16 1:4

Corresponding samples are given on page 157

[redacted] Book 1060 page 158 S. Phipps [redacted]

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

To Page No. 159

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by [redacted] Date [redacted]

Recorded by [redacted]

TITLE Endostatin Elisa

Project No. 414

Book N . 1060

From Page No. 158

[redacted]

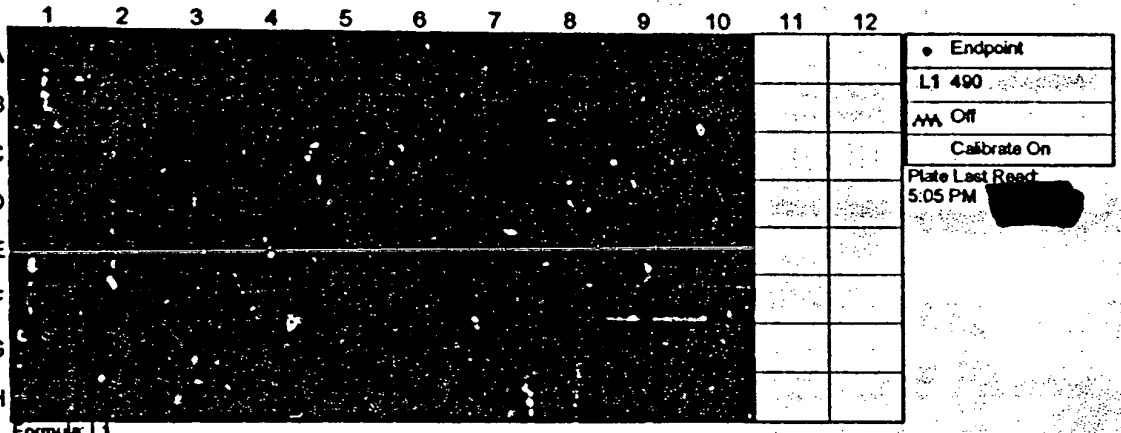
Book 1060 page 159

11/10/98

Experiment#1

[redacted] Sandra Phipps

Plate#1



Formula: L1

Data Mode: Absorbance

Book 1060 page 159

[redacted] Standards (ng/ml) S. Phipps

Sample	Concentration	BackCalcConc	Wells	Values	MeanValue	Std.Dev.	CV%
Std01	500.000	Range?	A1	0.188	0.200	0.017	8.5
		2194.026	B1	0.212			
Std02	125.000	106.785	C1	0.572	0.522	0.070	13.5
		143.302	D1	0.472			
Std03	31.250	27.646	E1	1.456	1.423	0.046	3.2
		29.730	F1	1.391			
Std04	7.810	8.838	G1	2.477	2.450	0.038	1.5
		9.459	H1	2.424			
Std05	1.950	2.729	A2	3.102	3.185	0.118	3.7
		1.279	B2	3.268			
Std06	0.000	Range?	C2	3.416	3.395	0.029	0.8
		0.263	D2	3.375			

Smallest standard value: 0.200

Largest standard value: 3.395

To Page No. 160

Witnessed & Understood by me, [redacted]

Date [redacted]

Invented by

Recorded by Sandra Phipps

Date [redacted]

Project No. 414
Book No. 1060 TITLE Human Exploration Elisa

From Page No. 159 [redacted]

UnknownsDilution

Book 1060 page 160
S. Phipps [redacted]

Sample	Wells	Values	R	Result	MeanResult	Std Dev.	CV%	Dilution	Adj Result
Un01	E2	3.329		0.723	0.723	0.000	0.0	2.0	1.446
	F2	3.437	R	Range?					
Un02	G2	3.365		0.370	0.868	0.704	81.1	4.0	3.471
	H2	3.258		1.366					
Un03	A3	1.370		30.454	28.174	3.224	11.4	2.0	56.349
	B3	1.515		25.895					
Un04	C3	2.211		12.186	13.385	1.696	12.7	2.0	26.770
	D3	2.050		14.584					
Un05	E3	1.852		18.040	17.818	0.314	1.8	2.0	35.635
	F3	1.875		17.596					
Un06	G3	0.796		66.751	69.915	4.475	6.4	2.0	139.830
	H3	0.746		73.079					
Un07	A4	3.206		1.825	1.338	0.690	51.6	2.0	2.675
	B4	3.318		0.850					
Un08	C4	3.358		0.444	0.796	0.497	62.5	2.0	1.591
	D4	3.283		1.147					
Un09	E4	3.341		0.609	1.725	1.578	91.5	2.0	3.450
	F4	3.089		2.841					
Un10	G4	3.324		0.770	0.635	0.192	30.2	2.0	1.269
	H4	3.352		0.499					
Un11	A5	0.281		421.473	377.655	61.969	16.4	2.0	755.309
	B5	0.306		333.836					
Un12	C5	0.412		180.290	172.435	11.110	6.4	2.0	344.869
	D5	0.434		164.579					
Un13	E5	3.229		1.625	1.163	0.654	56.2	2.0	2.326
	F5	3.331		0.700					
Un14	G5	3.382		0.185	0.661	0.674	101.9	2.0	1.323
	H5	3.284		1.138					
Un15	A6	3.341		0.613	0.613	0.000	0.0	2.0	1.226
	B6	3.414	R	Range?					
Un16	C6	3.242		1.507	0.848	0.932	110.0	2.0	1.695
	D6	3.381		0.188					
Un17	E6	3.328		0.730	0.638	0.129	20.3	2.0	1.276
	F6	3.347		0.547					
Un18	G6	3.219		1.715	3.031	1.861	61.4	2.0	6.063
	H6	2.920		4.347					
Un19	A7	2.161		12.900	11.724	1.663	14.2	4.0	46.897
	B7	2.335		10.549					
Un20	C7	2.685		6.602	6.333	0.380	6.0	4.0	25.332
	D7	2.738		6.061					
Un21	E7	2.672		6.730	7.377	0.914	12.4	4.0	29.506
	F7	2.550		8.023					
Un22	G7	1.419		28.806	29.212	0.575	2.0	4.0	116.849
	H7	1.394		29.619					
Un23	A8	3.209		1.804	1.451	0.498	34.3	4.0	5.806
	B8	3.288		1.099					
Un24	C8	3.307		0.930	2.401	2.080	86.6	4.0	9.602
	D8	2.972		3.871					
Un25	E8	3.189		1.973	1.505	0.662	44.0	4.0	6.020

Goto page 161

Witnessed & Understood by me, [redacted] Date [redacted] Invented by [redacted]

Recorded by [redacted]

From Page No. 160

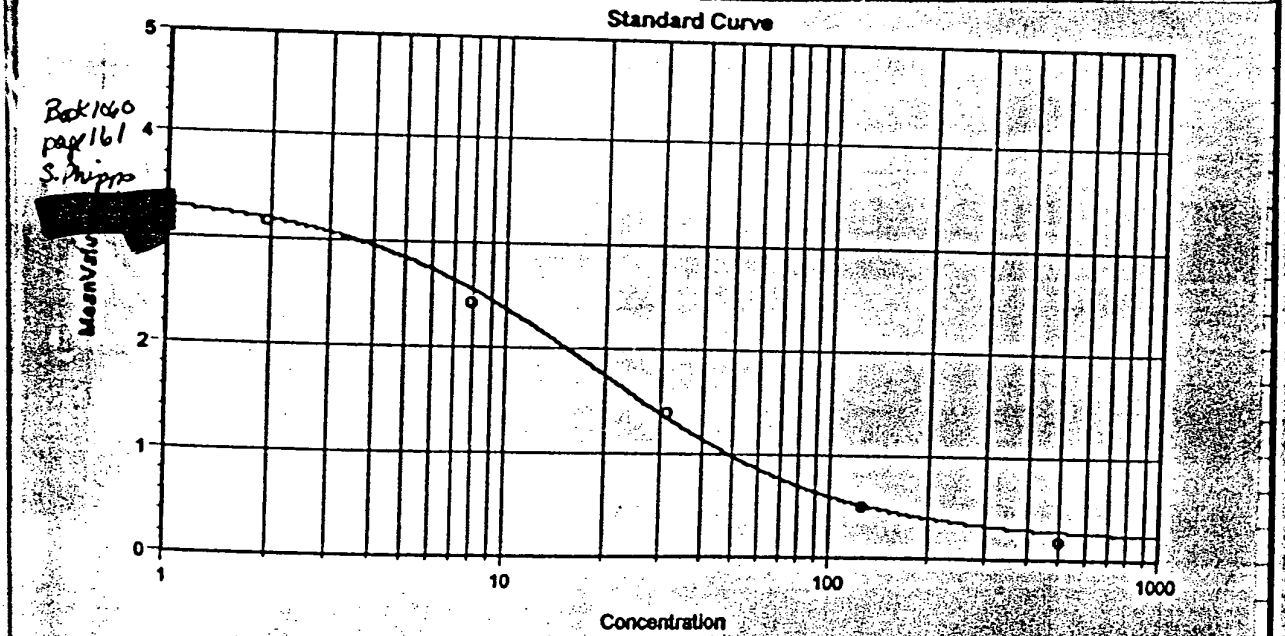
[redacted]

	F8	3.295	1.037					
Un26	G8	3.272	1.242	4.930	5.215	105.8	4.0	19.718
	H8	2.496	8.617					
Un27	A9	0.421	173.886	149.016	35.172	23.6	4.0	596.063
	B9	0.518	124.145					
Un28	C9	0.631	92.676	87.254	7.668	8.8	4.0	349.015
	D9	0.688	81.832					
Un29	E9	3.120	2.576	2.236	0.480	21.5	4.0	8.946
	F9	3.198	1.897					
Un30	G9	3.329	0.728	1.028	0.425	41.3	4.0	4.113
	H9	3.263	1.328					
Un31	A10	3.357	0.454	0.305	0.210	69.0	4.0	1.221
	B10	3.384	0.156					
Un32	C10	3.218	1.719	1.288	0.609	47.2	4.0	5.154
	D10	3.315	0.858					
Un33	E10	3.301	0.982	1.133	0.213	18.8	4.0	4.533
	F10	3.268	1.284					
Un34	G10	3.117	2.597	2.181	0.588	26.9	4.0	8.726
	H10	3.213	1.766					

[redacted]

Book 1060 pg# 161
S. Phipps

R - Outside standard range
Mean Adjusted Result: 77.18



Book 1060 pg# 161
S. Phipps

[redacted]

y=(A-D)/(1+(x/C)^B)+D:	A	B	C	D	R^2
Std (Standards: Concentration vs MeanValue)	3.395	1.178	19.115	0.2	0.988

Witnessed & Understood by me,

Date [redacted]

Invented by

Recorded by

Date [redacted]

From Page No. [redacted] Particles/ml of AV8MhandLx

Collected supernatant from AV4 human endostatin 4 well - 4th amplification made with 3 plasmid System (293 cells) 3.75 ug DNA + 1.67 ug DNA 9day pi.
Collected Sup from AV3 human endostatin - 3rd amplification made 3 plasmids in Clone 54 cells. 9day pi.
Collected 2 wells each of Mouse TPLi endostatin & human TPLi endostatin supernatant - from initial 3 plasmid transfection of AV4 on Clone 54 cells. (7day pi.) - Stored @ 4°C for ELISA testing

Harvested 9day amplified CVL from above samples AV4 human endo - 5 plasmid (293) 4th amplification on Clone 54 cells.
Harvested CVL from AV4 human endo - made 3 plasmid clone 54 9day pi.
Harvested initial CVL from transfection (7day post transfection) of AV4 human + mouse TPLi endostatin made in Clone 54 cells using lipofectamine + 3 plasmids.

Freeze thawed above CVLS 5X - re amplified 2nd AV4 human endo - 5th amplification on 10cm dish clone 54 cells added 6 ml of 3.75 ug amplified 1.67 ug DNA CVL AV4 human endostatin 5th amplification on 10cm dish clone 54 cells.

Amplified 1st time from original transfection CVL of mouse + human TPLi endo AV4 made in 3 plasmid clone 54 cells on 4 wells of clone 54 passage 14 cells. Added 0.5ml / well.

Split Chang cells p4 T150 1:10
Split 293 cells p24 T150 1:20

Took 2 (50ul) aliquots of AV8MhandLx Virus & 1 (100ul) & 1 (200ul) aliquot to look @ particles/ml & make Viral genomic DNA.

100ul Virus — 10min @ 56°C with shaking
900ul Viral concentrator buffer — 10min @ room temp.
Read 260nm OD

To Page No.

Witnessed & Understood by me. [redacted] Date [redacted] Invented by [redacted] Recorded by [redacted] Date [redacted]

TITLE Particles of AVBM hand LX (AV3 human endostatin Virus

Project No. 1114
Book No. 1060

From Page No. [redacted]

To make viral genomic DNA for LX
175 ul virus
175 ul 10x TES
7.5 ul proteinase K incubate @ 56°C 3½ hr with shaking
Phenol/Chloroform extract 1x; isoamyl/Chloroform extract 1x
Ethanol ppt. leave over weekend @ -20°C (1ml EtoH + 200 ul DNA.

OD readings for particles are below. I don't understand why 2nd aliquot of 10 ul gave such high OD reading. I will proceed particles on @ least 1 more aliquot & compare to these results.

BECKMAN DU-600

Date: [redacted]
Time: 12:58

Nucleic Acid
ReadSamples Method SaveClear Print Quit

Results file: A:\WORK_RES
Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No]

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1	0.0701	0.0670	1.0461	0.9559	0.0000	0.1751
2	0.0711	0.0679	1.0475	0.9546	0.0000	0.1778
3	0.2508	0.2535	0.9894	1.0107	0.0000	0.6270
4	0.2562	0.2575	0.9951	1.0049	0.0000	0.6406
5	0.0547	0.0533	1.0255	0.9751	0.0000	0.1367
6	0.0560	0.0523	1.0715	0.9332	0.0000	0.1401
7						

Book 1060
page 165
S. Phipps
[redacted]

OD reading of AVBM hand LX (AV3 human endostatin Virus
Lot #55P6-98

0.0706 x 10 x 1.12x10^12 particles/ml = 7.9x10^11

0.05535 x 10 x 1.12x10^12 particles/ml = 6.2x10^11

I did swipe tests room 110

Based on aliquot #1 + 3 the average particle/ml is

7.05x10^11 particles/ml

To Page No.

Witnessed & Understood by me, [redacted]

Date [redacted]

Invented by [redacted]

Recorded by [redacted]

Date [redacted]

TITLE AVBMhendLX Viral DNA/genomic DNA Isolation of
Project No. 414
Book No. 1060

From Page No. [redacted]

Mouse + Human TPLintron Endostatin

Finished Puregene genomic DNA isolation of mouse + human TPLi-endostatin and Endostatin transient transfections from page 163

Precipitated AVIBMhendLX viral genomic DNA from 165 → Centrifuged 5min. Washed 1X 70% EtOH. air dried pellet. Resuspended 50ul 1X TE.

Split S8 cells passage 25 to make 50 TCID50 plates (96 well plates)
Used 1 roller bottle
172 } 174.5x10⁴ cells/ml
177 }

5x10⁴ cells/ml x 1000 ml = 5x10⁷ cells / 174.5x10⁴ cells/ml | Need 28.65 ml cells |

+ 4ml dexamethasone
Plated 200ul/well for 1x10⁴ cells/well (96 well plate)

Plated 4 6 well dishes
3.5x10⁵ cells/well Need 8.75x10⁴ cells/ml x 110 ml = 9.62x10⁶ cells / 174.5x10⁴ cells/ml
Need 5.5ml of cells + 105 ml media + 0.33 ml dexamethasone

Split 1 T150 1:20

Sandina Phipps [redacted]

[redacted]

Ran PCR products 0.8% agarose gel 10ul of each run.
Lane 1 mwt λHindIII ΦXHaeIII
Lanes 2-19 → 1a thru 59 1a
(#1) #18
Gel picture page 166
Lane 20 - 1Kb ladder

The correct fragment ≥ 1Kb
Bottom gel broke next to molecular wt marker is 0.4ug pAVE1aLX2 d to the left of that 1.2ug pAVE1aLX2 + no DNA control well

To Page No. 168

Witnessed & Understood by me, [signature] Date [redacted] Invented by Date [redacted]
Recorded by Sandina Phipps

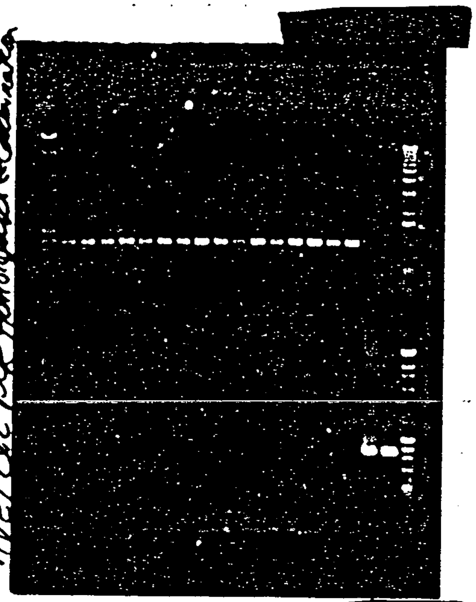
Project No. 414
Book No. 1060
TITLE PCR Amplification of Homologous Recombinants

From Page No. 167

[redacted] EXP 1-4. / Amplification of AV4 Human Endostatin

Lanes 18-19 top gel have SQ1a duplicates which should not amplify with these PCR primers YNC572/C1412

AV E1 C12 PCR Homologous Recombinants



Book1060 page 168 J. Phipps [redacted]

Collected 5th amplification CVL of AV4 human endostatin made (293 5 plaque) then transferred to Clone 54 for 4 amplifications.
Collected 10 cm plate - took 4 ml after centrifuging to run on human endostatin elisa kit (from page 164)
Froze CVL @ -70°C. The CVL was CPE @ 4 days post infection

To Page No.

Witnessed & Understood by me, [signature] | Date [redacted] | Invented by | Date [redacted]
Recorded by [signature]

TITLE E2... From Page N... 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 Witnessed & Un...

Project No. 414
Book No. 1060
TITLE TCID50 Titer of AV3BmhendLx Virus / Tissue Culture

From Page No. [redacted]

Split Hep 3B cells 2 T_{150} 1:2 & 1:20 pass 61 EMEM + 10% FBS
Split Chang cells T_{150} 1:40 pass 5 after freeze EMEM + 10% FBS
Split 293 T_{150} 1:40 pass 25
Split LS180 T_{150} 1:40 pass 4 after thaw

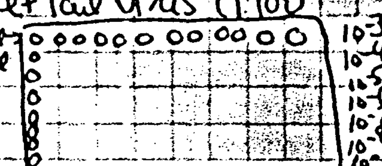
Split Clone 54 cells passage 15
104 }
86 } 88 x 10^4 cells/ml
74 }

Need 1×10^5 cells/ml x 26ml = 2.6×10^6 cells / 88×10^4 cells/ml
Need ~~2 3ml~~ 3ml of cells + 78ul dexamethasone

Need 4×10^5 cells / 10cm plate
4×10^5 cells/ml x 12ml = 4.8×10^6 cells / 88×10^4 cells/ml
5.45ml + 36ul dexamethasone

Split 2 T_{150} 1:30 put 90ul dexamethasone in 30ml
Split 1 T_{150} 1:20 to carry passage 15
Split Hela cells 1:50
Split Saos2 cells passage 2 from freeze T_{150} 1:10
Diana Moffat will freeze these cells Saos2 down today approx 10 vials

Made serial dilutions of AV3 BmhendLx virus Lot SSP6-98
Diluted virus 1:100 then made 10-fold dilutions to put down
96 well plate 990ul + 10ul virus (1:100)
Add 20ul/well with repeator



Did duplicate plates from 2 sets of serial dilutions

Also made 10ul aliquots of virus + 90ul concentration lysis buffer to repeat particle/ml calculation. Incubate 10min @ 56°C then @ room temp for 10min. Read 260nm OD reading. OD Readings were too low need to repeat.

To Page No.

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

TITLE Human Endostatin Assay for Lentivirus / Project No. 414 Book No. 1060

From Page No. [redacted] / AV4 + AV3 BMhendLX

Split Hep3 Balls 1:50 for Theresa Chen 1 ~~and~~ 2 T150 passage 62
Split Chang cells 1:50 T150 → passage 6.

Set up endostatin Elisa (human) Tested lentivirus supt for endostatin heparin purified AV3 BMhendLX virus + AV4 BMhendLX virus supernatants.

Made 1:2 & 1:4 dilutions for all samples except the heparin column purified AV3BMhendLX made 1:10, 1:50, 1:250, 1:500 dilutions

Followed protocol page 1059/106

For 1:2 use 200ul sample + 200ul diluent #1
For 1:4 use 100ul sample + 200ul diluent 1 + 100ul diluent 2
For 1:10 use 40ul purified AV3BMhendLX in 260ul diluent #1 100ul diluent #2
1:50 use 8ul + 92ul diluent extra
1:250 use 16ul of 1:10 + 84ul extra diluent #1
1:500 use 8ul of 1:10 + 92ul extra diluent #1

Samples

~~1 AV3 TPLi endLX supt transfection 3 plasmid S8 12 day p.i.~~ [redacted]
2 AV4 TPLi endLX Supt Clone 54 3 plasmid 7 day p.i. [redacted] 1st supt from transfection
9. AV4 TPLi BMhendLX Clone 54 3 plasmid 7 day p.i. [redacted] " " "
4 AV4 TPLi BMhendLX clone 54 5th round amplification (293 plasmid) 3.75ug 4th day p.i. [redacted]
3 AV4 human endo Clone 54 cells 3rd amp. 6b ~~made~~ 3 plasmid Clone 54 8 day p.i. [redacted]
6 AV3 TPLi endLX supt transfection 3 plasmid S8 12 day p.i. [redacted]
7 AV4 TPLi endLX 8 day p.i. supt from initial transfection 5 plasmid 293 [redacted]
8 AV3 TPLi BMhendLX sup transfection 5 plasmid S8 12 day p.i. [redacted]
~~9 AV4 TPLi BMhendLX supt after initial transfection 293 5 plasmid 8 day p.i.~~ [redacted]
10 Clone 54 / passage amp 4 AV4 human endostatin (5 plasmid 293) 9 day p.i.
11 (200ul) AV3 human endo [redacted] - dialyzed.

Chris Wysocki Set up lentivirus supernatants 8 samples (2 dilutions 1:2, 1:4) 8 samples are duplicates of one another

① AV4 BMhendLX 1.67ug DNA (293 5 plasmid 3rd amp [redacted] 9 day p.i.
5 AV4 TPLi BMhendLX supt after initial transfection 293 5 plasmid [redacted] 8 day p.i.

To Page No. 180

Witnessed & Understood by me, [signature] | Date [redacted] | Invented by | Recorded by [signature] | Date [redacted]

Project No. [illegible]
Book No. [illegible] TITLE [illegible]

From Page No. 172 [redacted]

17[illegible]

[illegible] spike [illegible] calibration curve [illegible] pg 172

[illegible] 5 [illegible]

[illegible] 5 [illegible] acetate

Make 1 [illegible] in H_2O 5 [illegible] sample + 245 [illegible] H_2O

[redacted] [illegible] Book 1060 pg 1[illegible] [illegible]

	1	2	3	4	5	6	7	8	9	10	11	12
A	3 ng/ml	1 ng/ml	#1 1:4	#3	#5	#7 1:4	#9 1:4	[illegible] 1:2			1 1:2	5 1:2
B	↓	↓	↓	↓	↓	↓	↓	↓			1 1:4	5 1:2
C	[illegible]	[illegible]	#2 1:2	#4 1:2	#6 1:2	#8 1:2	#10 1:2	# 1:50			2 1:2	6 1:2
D	↓	↓	↓	↓	↓	↓	↓	↓			2 1:4	6 1:4
E	3 ng/ml	[illegible]	#2 1:4	#4 1:4	#6 1:4	#8 1:4	#10 1:4	1:5			3 1:2	7 1:2
F	↓	↓	↓	↓	↓	↓	↓	↓			3 1:4	7 1:4
G	7 ng/ml	#1 1:2	#3 1:2	#5 1:2	#7 1:2	#9 1:2	X	# 1:500			4 1:2	8 1:2
H	↓	↓	↓	↓	↓	↓	X	↓			4 1:4	8 1:4

	1	2	3	4	5	6	7	8	9	10	11	12
A	[redacted]										Book	
B											1060	
C											page 130	
D											S. [illegible]	
E											[redacted]	
F												
G												
H												

To Page No. 181

Witness [illegible] [redacted] Date

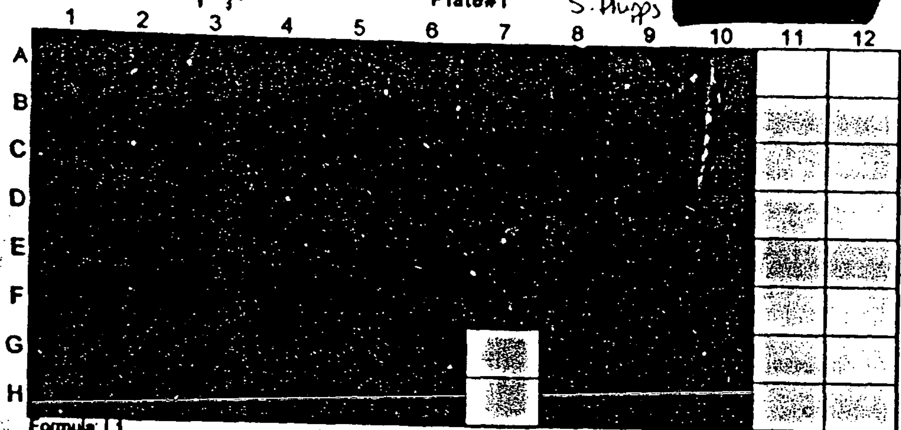
Recorded by [illegible] [redacted]

TITLE Human Endostatin Assay

Project No. 414
Book No. 1060

From Page No. 180 [redacted]

Book1060 page 181 Plate#1 S. Phipps [redacted]



UnknownsDilution Book1060 page 181 Sandra Phipps [redacted]

Sample	Wells	Values	R	Result	MeanResult	Std.Dev.	CV%	Dilution	Adj.Result	
Un01	E2	2.874	R	Range?	0.231	0.000	0.0	4.0	0.925	media
	F2	2.768		0.231						
Un02	G2	2.501		2.503	1.434	1.512	105.4	2.0	2.869	1 AV... 1.67μg
	H2	2.752		0.365						9dy 3'damp.
Un03	A3	3.090	R	Range?	Range?	Range?	Range?	4.0	Range?	1
	B3	2.830	R	Range?						
Un04	C3	2.877	R	Range?	Range?	Range?	Range?	2.0	Range?	2 AV... clone54
	D3	2.984	R	Range?						1st ... transfection
Un05	E3	2.502		2.490	2.490	0.000	0.0	4.0	9.960	2
	F3	2.866	R	Range?						
Un06	G3	2.623		1.439	1.439	0.000	0.0	2.0	2.878	3
	H3	2.795	R	Range?						AV4 ... 3'damp
Un07	A4	2.917	R	Range?	Range?	Range?	Range?	4.0	Range?	3 clone54/clone54
	B4	2.887	R	Range?						
Un08	C4	0.313		263.297	274.047	15.203	5.5	2.0	548.094	4 469ng/ml
	D4	0.303		284.797						AV4 ... 5'damp
Un09	E4	0.536		97.567	97.508	0.083	0.1	4.0	390.033	4 293 3.75μg (10cm dish)
	F4	0.537		97.450						
Un10	G4	2.583		1.780	2.337	0.788	33.7	2.0	4.674	5
	H4	2.458		2.894						AV4... 5plasmid
Un11	A5	2.954	R	Range?	Range?	Range?	Range?	4.0	Range?	5 293
	B5	2.863	R	Range?						
Un12	C5	2.853	R	Range?	Range?	Range?	Range?	2.0	Range?	6
	D5	2.825	R	Range?						AV3... 3plasmid
Un13	E5	2.830	R	Range?	Range?	Range?	Range?	4.0	Range?	6 S8
	F5	3.010	R	Range?						
Un14	G5	2.808	R	Range?	Range?	Range?	Range?	2.0	Range?	7
	H5	2.805	R	Range?						AV4... 5plasmid
Un15	A6	2.958	R	Range?	Range?	Range?	Range?	4.0	Range?	7 293
	B6	3.013	R	Range?						

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Witnessed & Understood by me, [signature]

Date [redacted]

Invented by

Recorded by Sandra Phipps [redacted]

Date [redacted]

Project No. 414

Book No. 1060 TITLE Human Endostatin Assay

From Page N. 181

[redacted]

Un16	C6	2.685	0.916	0.715	0.285	39.9	2.0	1.430
	D6	2.734	0.513					
Un17	E6	2.664	1.090	0.725	0.516	71.2	4.0	2.900
	F6	2.752	0.360					
Un18	G6	0.550	93.705	91.411	3.244	3.5	2.0	182.822
	H6	0.568	89.117					
Un19	A7	0.990	36.823	37.318	0.701	1.9	4.0	149.273
	B7	0.974	37.814					
Un20	C7	0.808	51.133	51.723	0.835	1.6	2.0	103.446
	D7	0.796	52.314					
Un21	E7	1.320	22.139	18.309	5.417	29.6	4.0	73.234
	F7	1.616	14.478					
Un22	A8	0.219	606.772	688.457	167.322	24.3	10.0	6884.573
	B8	0.238	570.143					
Un23	C8	0.753	57.136	53.852	4.645	8.6	50.0	? 2692.576
	D8	0.813	50.567					
Un24	E8	1.258	24.252	23.207	1.478	6.4	250.0	5801.704
	F8	1.320	22.161					
Un25	G8	1.609	14.613	15.496	1.249	8.1	500.0	7748.092
	H8	1.530	16.380					

8 (2.165 ng/ml) AV3TPLiBMHandLX
8 3 plasmid S8
9 (1.66 ng/ml) AV4 TPLiBMHand LX S4
9 3 plasmid
10 (8834) AV4 passage 4
10 Sphaerid 293 clone 54
11 AV3BMHandLX
11 pooled
- dilution made wrong
11
11 Avg. 6811
6.8 ug/ml

[redacted] 4:59:40 PM HUENDO112398SSP.pds

Chris Wysocki's samples
Lentivirus supernatants

Un26	A9	2.699	1:2	0.802	0.802	0.000	0.0	2.0	1.604
	B9	3.066	R 1:4	Range?					
Un27	C9	2.811	R 1:2	Range?	Range?	Range?	Range?	4.0	Range?
	D9	2.958	R 1:4	Range?					
Un28	E9	1.428	1:2	18.945	12.214	9.519	77.9	2.0	24.428
	F9	2.204	1:4	5.483					
Un29	G9	1.561	1:2	15.671	10.542	7.254	68.8	4.0	42.167
	H9	2.211	1:4	5.412					
Un30	A10	2.885	R 1:2	Range?	Range?	Range?	Range?	2.0	Range?
	B10	2.962	R 1:4	Range?					
Un31	C10	3.142	R 1:2	Range?	Range?	Range?	Range?	2.0	Range?
	D10	2.986	R 1:4	Range?					
	E10	2.975	R 1:2	Range?					
	F10	3.007	R 1:4	Range?					
Un32	G10	3.131	R 1:2	Range?	Range?	Range?	Range?	4.0	Range?
	H10		R						

Book 1060 page 182 S Phipps

Book 1060 pg 182 Standards (ng/ml) [redacted] S Phipps

Sample	Concentration	BackCalcConc	Wells	Values	MeanValue	Std.Dev.	CV%
St01	500.000	Range?	A1	0.145	0.176	0.043	24.5
		1123.306	B1	0.206			
St02	125.000	133.936	C1	0.444	0.496	0.074	15.0
		94.118	D1	0.549			
St03	31.250	24.320	E1	1.256	1.131	0.177	15.6
		35.833	F1	1.007			
St04	7.810	8.419	G1	1.970	1.940	0.043	2.2
		9.300	H1	1.909			
St05	1.950	0.927	A2	2.684	2.559	0.177	6.9
		3.117	B2	2.434			
St06	0.000	Range?	C2	2.956	2.793	0.230	8.2
		1.370	D2	2.631			

To Page No. 183

[redacted] Recorded by [redacted] Sandra Phipps

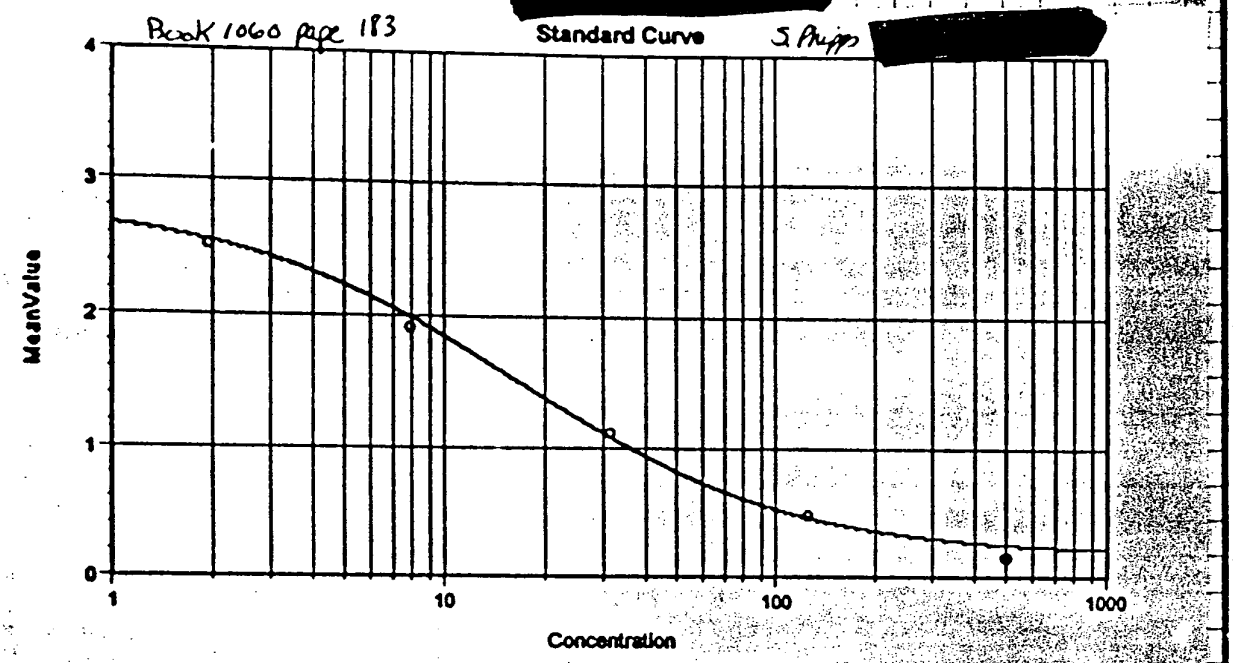
TITLE Human Endostatin Assay

Project No. 414

Book No. 1060

From Page N 182

[redacted]



The repair purified AV3BMhendLX virus 6.8 ug/ml

The AV4BMhendLX virus amplifications
AV4BMhendLX amplification passage (4) 88.34 ng/ml
AV4BMhendLX amplification passage (5) 469 ng/ml

The AV4BMhendLX clone 54 (3 plasmid) 7 day p.i. from original transfection Supt
now has 166 ng/ml of human endostatin
None of other AV3 & AV4 PI virus endostatin virus clones produced much endostatin
Sandra Phipps [redacted]

[redacted]

Split 58 cells passage 28 1:50
" SaOS2 cells passage 3 1:50
" LS180 cells passage 3 1:50
" Hela cells 1:50
Threw away 293 cells because of high passage
Gave the Split Clone 54 Cells to me.

To Page No. 184

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by Sandra Phipps Date [redacted]

Recorded by Sandra Phipps

Project No. 414
Book No. 1060 TITLE Collecting Amplified CVL of AV4 & AV3 TPLi

From Page No. [redacted]

BMH and LX

Titer of AV3 BMH and LX virus

Split 293 cells 1 15 complete 1:10, 1:20 (2T150) passage 10 from Dawn Kayda.
Split 58 cells passage 12: Gene Line Split 1 15 complete into 6 well dishes @ 4x10^5 cells/well.

Collected supt and CVL from AV4TPLi and Lx & AV4TPLiBMH and Lx 1st Amplification Clone 54 cells (293 Spleen d system) - 10 day p.i.
Collected supt and CVL from AV3TPLi and Lx & AV3TPLiBMH and Lx 1st amplification 58 cells (3 plasmid 58 cells) 11 day p.i.
Froze CVL @ -70°C after Snap freezing.
Stored supt @ 4°C.

Read TCID50 titers of AV3 BMH and LX Virus

Book 1060 page 186
S. Phipps

Read [redacted] 1:100

Sample: AV3 BMH and LX Virus (B)

	1	2	3	4	5	6	7	8	9	10	11	12
B	+	+	+	+	+	+	+	+	+	+	+	+
C	+	−	−	−	+	+	+	+	−	+	+	+
D	+	−	−	−	−	−	−	+	−	−	−	−
E	+	−	−	−	−	−	−	−	−	−	−	−
F	+	−	−	−	−	−	−	−	−	−	−	−
G	+	−	−	−	−	−	−	−	−	−	−	−
H	−	−	−	−	−	−	−	−	−	−	−	−

BX

[redacted] 1:100

Sample: AV3 BMH and LX Virus A

	1	2	3	4	5	6	7	8	9	10	11	12
B	+	+	+	+	+	+	+	+	+	+	+	+
C	+	+	−	+	+	−	+	+	+	+	+	+
D	+	−	−	−	+	−	−	−	−	−	−	−
E	−	−	−	+	−	−	−	−	−	−	−	−
F	−	−	−	−	−	−	−	−	−	−	−	−
G	−	−	−	−	−	−	−	−	−	−	−	−
H	−	−	−	−	−	−	−	−	−	−	−	−

7.97x10^8
9.65x10^8
8.81x10^8

Titer of B - 7.97x10^8
A - 9.65x10^8
} Avg. titer 8.8x10^8 PFU/ml

To Page No.

Witnessed & Understood by me, [redacted]

Date [redacted]

Invented by

Record d by [redacted]

Date [redacted]

TLE Human Endostatin (His Endo)

From Page No. 9

[redacted] 1) use Hi Trap Heparin (pharmacia Biotech #17-0406-01) Column to process binding Sup. (room temperature).

wash w/ 10 ml of PBS (using 10ml syringe)

↓

Loaded 100ml of Supernatant.

↓

wash w/ 10ml of wash buffer (50mM Tris-Cl pH 7.5
100mM NaCl

↓

Eluted the Heparin bound protein with 10ml Elute Buffer (50mM Tris-Cl pH 7.5
1M NaCl
20% glycerol)

↓

collected fractions at 1ml/tube for 10 tubes

↓

protein Assay to determine protein Conc.

Assay type: General Ratio and Concentration Units: ug/ml
Formula setup: TIBB modified Saul Background Correction: [No]
Sampling device: None Concentration: [Yes]
Read average time: 0.50 sec Peak Pick: [No]

Sample ID	abs 280.0 nm	abs 280.0 nm	280.0 nm / 280.0 nm	Protein ug/ml	Nucleic Acid ug/ml	
1	2.0230	2.0344	0.9953	1.0047	0.0000	0.0575
2	2.7017	2.6965	1.0022	0.9240	0.0000	0.7544
3	0.5550	0.4922	1.1276	0.8866	0.0000	1.3075
4	0.3230	0.2772	1.1652	0.8402	0.0000	0.8240
5	0.1705	0.1570	1.1372	0.8793	0.0000	0.4463
6	0.1206	0.1206	1.0491	0.9532	0.0000	0.3164
7	0.1421	0.1297	1.0952	0.9131	0.0000	0.3668
8	0.0698	0.0770	0.9024	1.1333	0.0000	0.1719
9	0.0518	0.0670	0.7706	1.2978	0.0000	0.1281
10	0.0327	0.0567	0.6455	1.5491	0.0000	0.0818

pooled tube #1 & #2, keep in 4°C.

To Page No. 10

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by [redacted] Date [redacted]

Recorded by [redacted]

Project No. 414
Book No. 1127 TITLE AV3hEndo

From Page No. 9

[redacted] Dialysis (desalt) of AV3hEndo

using dialysis cassette - from pierce Slide-A-Lyzer (3.5kD) # 98061975

dialysis 200ul of pooled #1 & #2 (samp 9) in 500ml of PBS, cold Room X 2hrs

[redacted] protein Assay of AV3hEndo

		●	①	②	③	④	⑤	⑥	⑦	8	9	10	11
0.8ml H_2O	ul		800	800	800	800	800	800	800	800	800	800	800
0.2ml Bio-Rad protein Assay dye #500-0006	ul		200	200	200	200	200	200	200	200	200	200	200
BSA (1ug/ul)	ul		0	2	4	6	8	10	15	2 Endo	4	2	4

↓

Transfer 200ul to 96-well plate, each sample for 3 wells

↓

Read ABS at 595 nm

Experiment#1
#1 & #2 pooled PAV3hEndo desalted PAV3hEndo

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001											
B	-0.002											
C	0.001											
D	0	2λ	4λ	6λ	8λ	10λ	15λ	2λ	4λ	2λ	4λ	
E												
F												
G												
H												

Bio-Rad Ins λ

● Endpoint
L1 595
AA Off
Calibrate On
Plate Last Read: 12:06 PM [redacted]

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by [redacted]

Recorded by [redacted] Date [redacted]

From Page No. 10

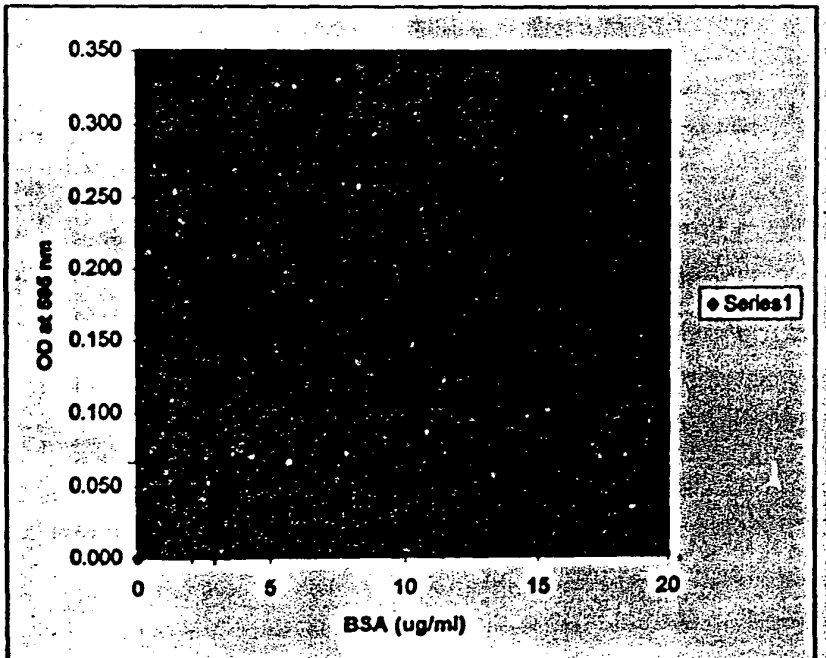
BSA(1mg/ml)	well 1	2	3	Average	
0	0.001	-0.002	0.001	0.000	
2µl :	0.058	0.065	0.058	0.060	
4µl :	0.106	0.105	0.103	0.105	
6µl :	0.119	0.119	0.112	0.117	
8µl :	0.19	0.191	0.186	0.189	
10µl :	0.265	0.26	0.268	0.264	
15µl :	0.334		0.294	0.314	
hEndo					
2ul u1	0.133	0.13	0.133	0.132	0.066
4ul u1	0.253	0.247	0.242	0.247	0.062
8ul u2 >desalt	0.101	0.102	0.098	0.100	0.050
4ul u2	0.165	0.165	0.157	0.162	0.041

Keep in -70°C

3mg/ml — hEndo pooled #1 & 2

2mg/ml (diluted) — desalted hEndo

Keep in 4°C



To Page No. 12

Witnessed & Understood by me, [redacted] Date

Invented by

Recorded by [redacted] Date

Project No. 414
Book No. 1127 TITLE AV3hEndo

From Page No. 11 SDS-page : 4~20% pre-cast gel (Bio-Rad)

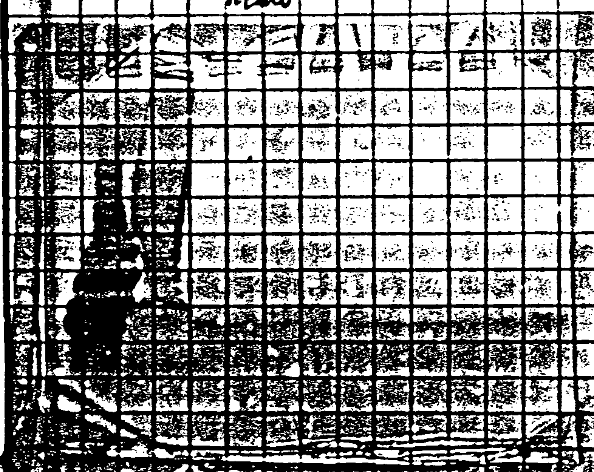
[redacted] AV3hEndo : desalted ~2mg/mL

Samples boiling : 105ul dye + 105ul PAV3endo
90°C x 3min

Gel I (For Commassie blue Staining) 120V for ~1 hr

Gel II Load 20ul each Lane
For transfer PVDF 0.2u filter (Schleicher & Schuell)
Transfer at 100V for 1hr

hEndo



marker: cuprotein midrange blue prestained protein marker (cat 7402)

Transfer procedure:
Soak PVDF membrane in methanol for a while
↓
Then Soak in western Transfer Buffer (keep in 4°C)
↓
TransferBlot (Bio-Rad)
See manual p7
gray panel + sponge + filter paper + gel + PVDF membrane (no bubbles in each layer)
↓
After transfer, the membrane will be stained with 0.1% Commassie blue R250 in 40% methanol and 1% ~~[illegible]~~ acetic acid for 2min; destain in 50% methanol
wash c ddH2O, 15 min per wash x4 times

SDS-gel
↓
Take the SDS-page gel, wash with ddH2O x 3 times
↓
Commassie stain (Pierce #980/2460) for 1hr
↓
wash with ddH2O

Witnessed & Understood by me, Date [redacted] Invented by
Recorded by Date [redacted]

From Page No. 12

hendo PVDF Transfer

↓

Send protein (AV3hendo) to Midwest Analytical, INC for protein Sequence

To Pag No.

Witnessed & Understood by me. [redacted] Invented by [redacted]

Recorded by [redacted]

From Page No. [redacted]

[Objectives]: To determine the cleavel product product from A-3 hEndo transduced Sf cells.

[Experiments]: Qin Li processed the conditioned medium of A-3 hEndo transduced Sf cells with Hepain Sepharose. Loaded the Hepain bound fraction pool on SDS-PAGE. The proteins were transferred to PVDF membrane and stained with Coomassie blue. The PVDF blot was sent to Midwest Analytical, Inc. for N-terminus peptide sequence analysis.

[Results]:

MIDWEST ANALYTICAL, INC.
[redacted] South Tower Square
St. Louis, MO 63123

[redacted] Notebook 1850 Page 129

Dr. Thomas Chen
Genetic Therapy, Inc.
99 Firefield Road
Gaithersburg, MD 20878

Dear Thomas:

Here are the chromatograms from the 20K band. The PTH standard was 20 pmol.

The primary sequence was APQQEALAMS.

The two secondary sequences matched your sequence, but started at different positions (LAMS... and MSHR...).

Please call me at 1-800-481-0790 if you have any questions about this run. Thanks again.

Sincerely,

David W. McCourt
[redacted]

Cheng-T. Chen

MIDWEST ANALYTICAL [redacted] South Tower Square St. Louis, MO 63123
David McCourt 800-481-0790

Dr. Thomas Chen, Genetic Therapy

Sample: 20K [redacted]
Two bands were loaded for 10 residue N-terminal analysis.

File: A3123 [redacted]

1	A	ST	N	L	A
2	P	A	S	K	
3	Q	N	N	P	
4	Q	S	S	K	
5	E	N	D		
6	A	R	F		
7	L	D	Q		
8	A	F	P / A		
9	M	Q	V		
10	S	P	L	A?	

E3 = 1.3 pmol D5 = 0.7 pmol

Thomas,

There was an unambiguous primary sequence and two secondary sequences.

The secondary components matched your sequence, but they started at different positions. The end of this sequence looks like QPV instead of QAG. I did not observe any increase in G at residue #9.

Cheng-T. Chen

Witnessed & Understood by me, M. O'Reilly Date [redacted]

Invented by Date

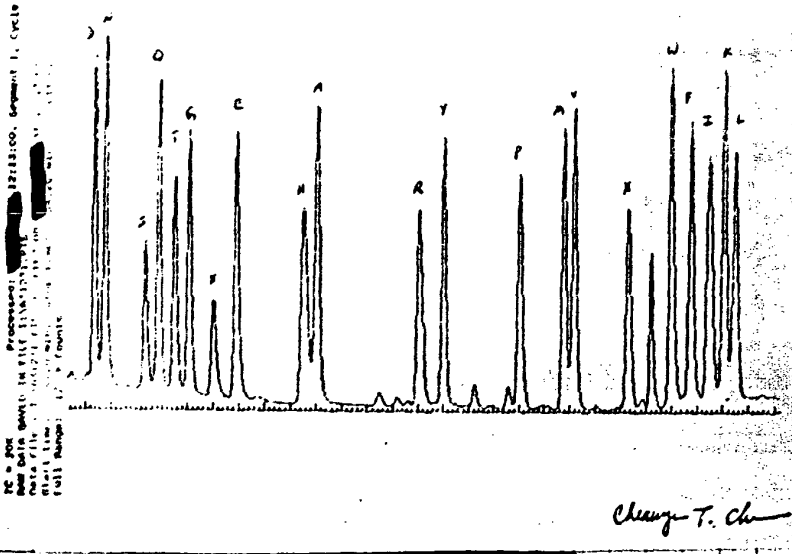
Recorded by Cheng-T. Chen [redacted]

Project No. [redacted] 414

130 Book No. 1080 TITLE N-terminus peptide sequence analysis of secreted human G-actin

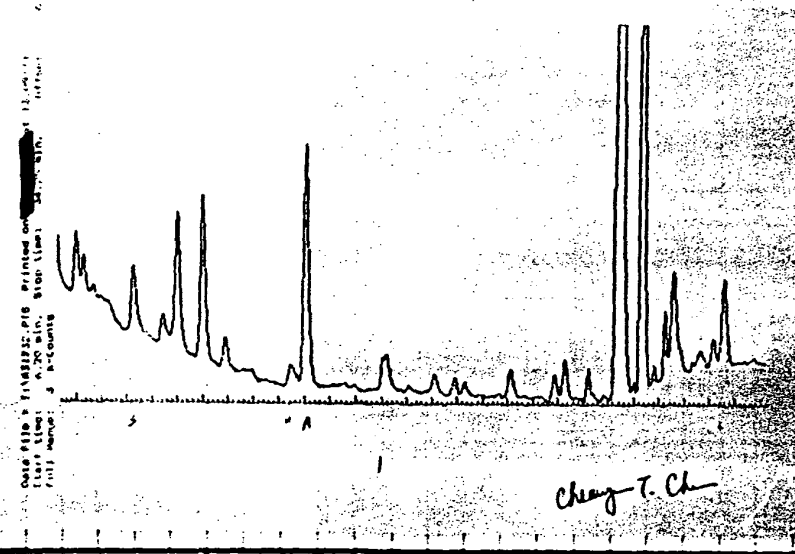
From Page No. [redacted]

Notebook 1080 page 130



Cheng-T. Chen

Notebook 1080 page 130



Cheng-T. Chen

To Page No. 131

Witness d & Und rstood by me,	Date	Inv nted by	Date
M. O'Reilly	[redacted]	Recorded by Cheng-T. Chen	[redacted]

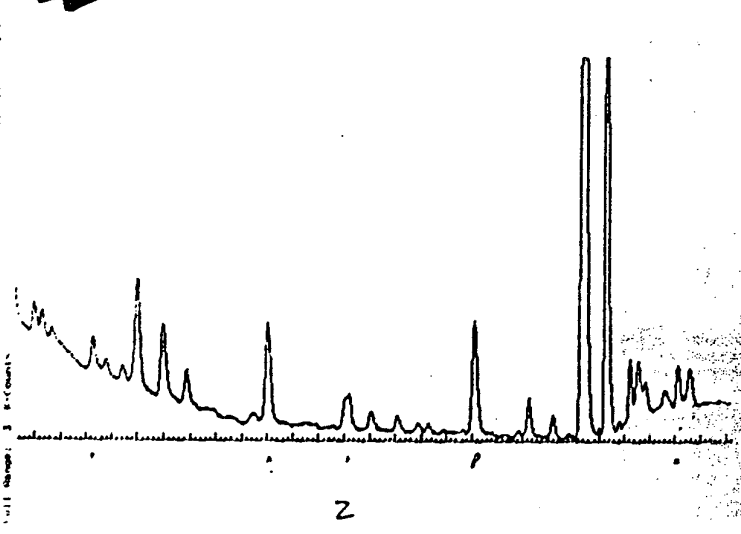
TITLE N-terminus peptide sequence analysis of secretin human intestinal Project No. 414

Book No. 1080

From Page No. 130

[redacted]

Notebook 1080 page 131

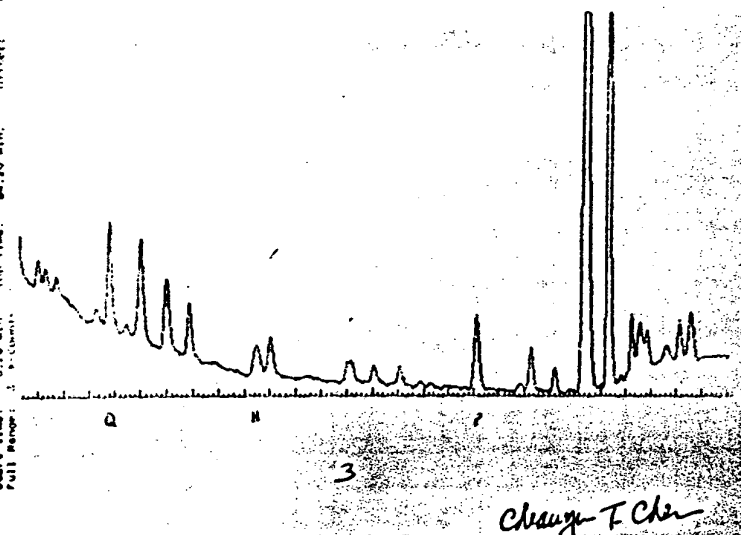


2

Cheung-T. Chen

[redacted]

Notebook 1080 page 131



3

Cheung-T Chen

To Page No. 132

Witnessed & Understood by m. Date [redacted]

M. O'Reilly

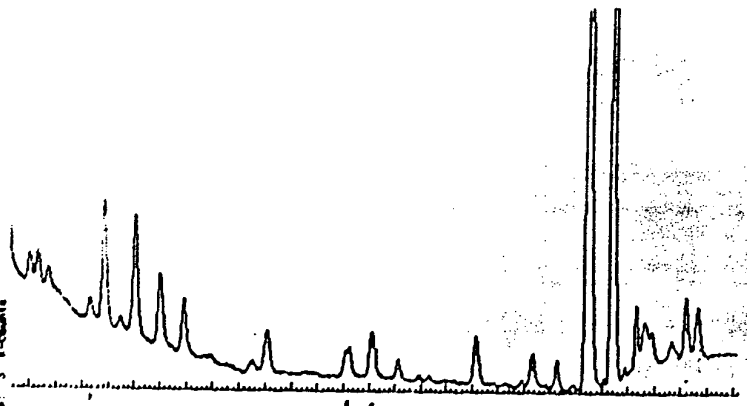
Invented by Date [redacted]

Recorded by Cheung-T. Chen

Project No. 414
Book No. 1880
TITLE N-terminus peptide sequence analysis of secreted human endostatin

From Page No. 13

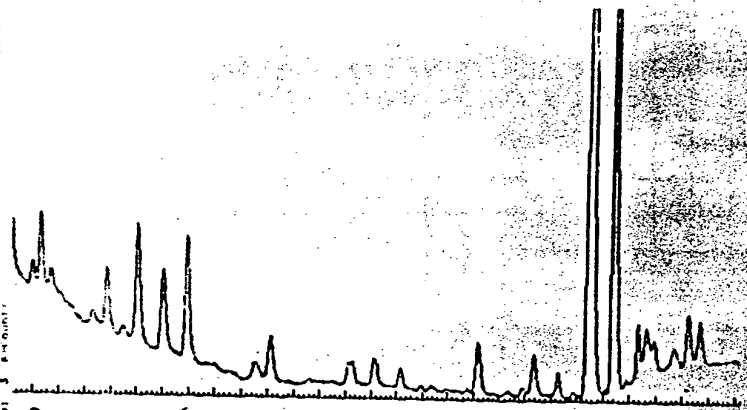
[redacted] [redacted] Notebook 1880 Page 132



4

Cheanyun T. Chen

Jan 4, 99 Notebook 1880 Page 132



5

Cheanyun T. Chen

To Page No. 133

Witnessed & Understood by me, M. O'Leilly Date [redacted]

Invented by Date

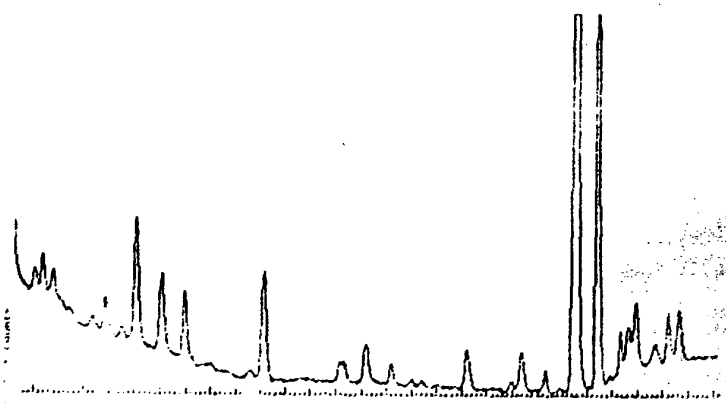
Recorded by Cheanyun T. Chen [redacted]

TITLE N-terminus peptide sequence analysis of human Endostatin Project No. 414
Book N . 1080

From Page No. B2

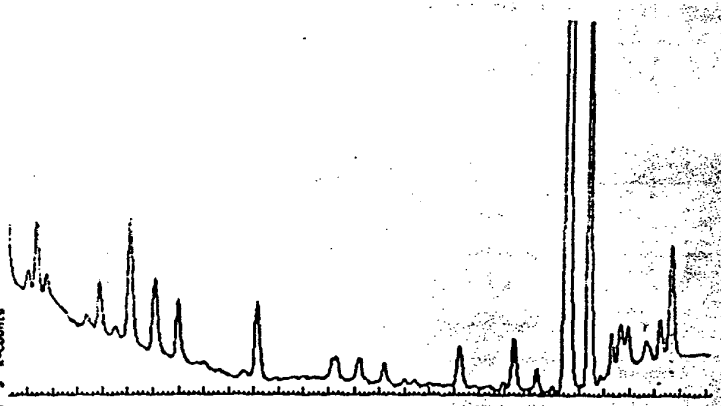
[redacted]

Notebook 1080 page 133



Cheunyu T. Che

Jan. 4, 99 Notebook 1080 page 133



Cheunyu T. Che

To Page No. 134

Witnessed & Understood by me, M. O'Reilly

Date [redacted]

Invented by

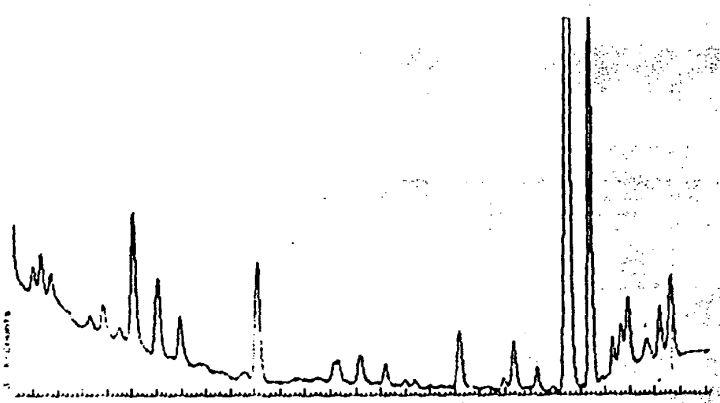
Date [redacted]

Recorded by Cheunyu T. Che

Project No. 414
Book No. 1080 TITLE N-terminus peptide sequence analysis of secreted human [illegible]

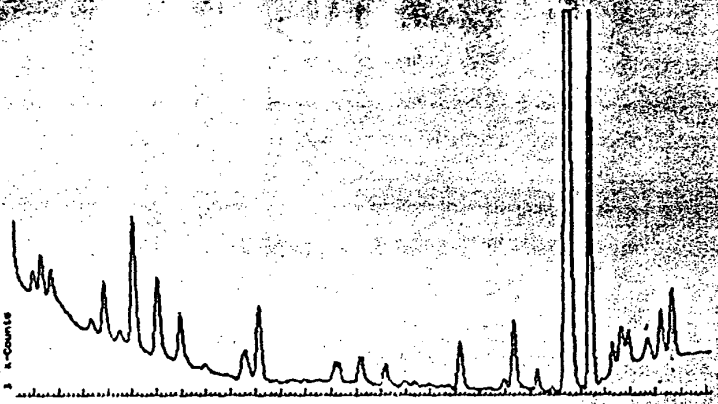
From Page No. 133

[redacted] Notebook 1080 page 134



Cheuyan T. Che

[redacted] Notebook 1080 page 134



Cheuyan T. Che

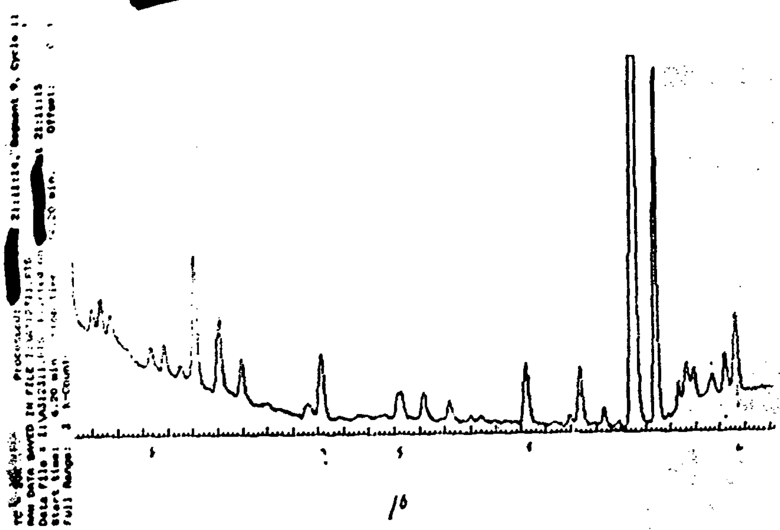
T Page No. 85

Witn s d & Understood by me.	Date	Invented by	Date
M. O'Reilly	[redacted]	Record d by Cheuyan T. Che	[redacted]

TITLE N-terminus peptide sequence analysis of secreted human Endostatin Project No. 414 Book No. 1080

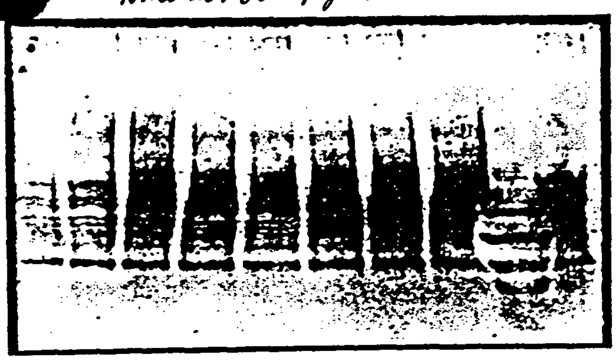
From Page No. 134

[redacted] Notebook 1080 page 135



Cheauyun T. Che

[redacted] Notebook 1080 page 135



50%
25%
25%

MRAWIFFLLCLAGRALAAPQQEALAHSHRDFQPVL----------

Human BM40 Signal Human Endostatin

Cheauyun T. Che

[Summary]
The sequence result showed expected peptide sequence which matched well with the predicted sequence. However, the cleavage site occur at 3 different locations. Whether or not the additional residues from signal peptide affect function will need to be tested.

T Page No.

Witnessed & Understood by me,	Date	Invented by	Date
M. O'Reilly	[redacted]	Recorded by Cheauyun T. Che	[redacted]